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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/720,177	11/25/2003	Jun Nakamura	US-110	6388
38108	7590	08/19/2009		
CERMAK KENEALY VAIDYA & NAKAJIMA LLP			EXAMINER	
ACS LLC			RAMIREZ, DELIA M	
515 EAST BRADDOCK ROAD				
SUITE B			ART UNIT	
ALEXANDRIA, VA 22314			PAPER NUMBER	
			1652	
			NOTIFICATION DATE	
			DELIVERY MODE	
			08/19/2009	
			ELECTRONIC	

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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**BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES**

Application Number: 10/720,177
Filing Date: November 25, 2003
Appellant(s): NAKAMURA ET AL.

Shelly Guest Cermak
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 5/8/2009 appealing from the Office action mailed 10/16/2008.

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(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments

The appellant's statement of the status of amendments contained in the brief is correct.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

EP 1229121 A2	Nakamura et al.	8-2002
WO 01/00843	Pompejus et al.	1-2001
EP 1 108 790	Nakagawa et al.	6-2001

Jakoby et al. "Isolation of the *Coryneform glutamicum* glnA gene encoding glutamine synthetase I" FEMS Microbiology Letters, vol 154 (1997), pp. 81-88

Jakoby et al., GenBank accession number Y13221 (1997)

Duran et al. "The role of glutaminase in *Rhizobium etli*: studies with a new mutant" Microbiology, vol 141 (1995), pp. 2883-2889

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Nakagawa et al., GenBank accession number AX127151 (2001)

Witkowski et al., "Conversion of a β -ketoacyl synthase to a malonyl decarboxylase by replacement of the active-site cysteine with glutamine" *Biochemistry*, vol 38 (1999), pp. 11643-11650

Seffernick et al., "Melamine deaminase and atrazine chlorohydrolase: 98 percent identical but functionally different" *Journal of Bacteriology*, vol 183, no. 8 (2001), pp. 2405-2410

Branden, C and J. Tooze, "Introduction to protein structure", Garland Publishing Inc., New York (1991), pp. 247

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 112, second paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1, 4-5, 12-16, 18-21 remain rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1, 5 and 16 (claims 4, 12-15, 18-21 dependent thereon) are indefinite in the recitation of "95% or more homologous to SEQ ID NO: X" for the following reasons. The term is unclear and confusing in the absence of a definition providing the intended meaning of the term or the intended parameters required to determine the required homology value. As stated in previous Office actions, while terms "homology" and "identity" are sometimes used interchangeably in the art, these terms are not equivalent. The calculation of sequence homology takes into consideration the type of mismatches such that even mismatches contribute to the % homology value, whereas mismatches are given no weight when calculating the % identity value, i.e., only matches contribute to the % identity value. Since (1) there is no indication in the specification that the intended meaning of the term "homology" is "identity", (2) it is

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clear from Appellant's responses that the intended meaning of the term is not identity, and (3) the specification does not provide the specific parameters/methods intended in the calculation of sequence homology (e.g., PAM matrices), one of skill in the art cannot determine the scope of the term "95% homologous" because a percent sequence homology value for a set of sequences is variable depending on the parameters used in the calculation and Appellant has not set forth the intended method/parameters for that calculation. One could have a nucleic acid sequence which is 95% sequence homologous to a reference sequence based on a particular set of parameters/matrix, and at the same time that same nucleic acid sequence being not 95% sequence homologous to the reference sequence if another set of parameters/matrix is used in the calculation. For example, using the scoring table (matrix) IDENTITY_NUC and parameter values Gapop = 10.0 and Gapext = 1.0, the polynucleotide of GenBank accession number BA000036_26 is 95.4 % sequence homologous to the polynucleotide of SEQ ID NO: 1. If the same calculation is carried out with the same scoring table and parameter values Gapop = 10.0 and Gapext = 0.1, the polynucleotide of GenBank accession number BA000036_26 is 96.1 % sequence homologous to the polynucleotide of SEQ ID NO: 1. For the reader's convenience, the cited alignments (provided to Appellant with the Office action mailed on 10/16/2008) are shown below. As indicated in the Office action mailed on 10/16/2008, the Scoring Table and parameters are shown first and homology is indicated as Query Match immediately prior to the alignment.

GenCore version 6.2.1

Copyright (c) 1993 - 2008 Bioceleration Ltd.

OM nucleic - nucleic search, using sw model

Run on: August 6, 2008, 16:09:37 ; Search time 15127 Seconds
(without alignments)
11364.178 Million cell updates/sec

Title: US-10-720-177-1
Perfect score: 2100
Sequence: 1 cacaaaatccggcgaaatcca.....ttgggattaagtgcctgcag 2100

Scoring table: IDENTITY_NUC
Gapop 10.0 , Gapext 0.1 <<<<<<<<

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Searched: 9588671 seqs, 40929980300 residues

Total number of hits satisfying chosen parameters: 19177342

Minimum DB seq length: 0

Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%

Maximum Match 100%

Listing first 45 summaries

Database : GenEmbl:*
 1: gb_env:*
 2: gb_pat:*
 3: gb_ph:*
 4: gb_pl:*
 5: gb_pri:*
 6: gb_ro:*
 7: gb_sus:*
 8: gb_sy:*
 9: gb_un:*
 10: gb_vl:*
 11: gb_ov:*
 12: gb_in:*
 13: gb_om:*
 14: gb_ba:*
 15: gb_htg1:*
 16: gb_htg2:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	% Query					Description
	Score	Match	Length	DB	ID	
	1	2100	100.0	2100	2	CQ817719 Sequence
	2	2100	100.0	2100	2	DD028154 Method fo
c	3	2018.2	96.1	110000	14	BA000036_26 Continuation (27 o <<<
c	4	2018.2	96.1	349136	14	BX927155 Corynebac
c	5	2018.2	96.1	349980	2	AX127151 Sequence
c	6	1977.9	94.2	110000	14	AP009044_26 Continuation (27 o
	7	1589.1	75.7	1629	2	BD164926 Novel pol
	8	1589.1	75.7	1629	2	AX122809 Sequence
	9	1436.1	68.4	1468	2	AX764345 Sequence
	10	1436.1	68.4	1468	2	EA032755 Sequence
c	11	884.2	42.1	110000	14	BA000035_25 Continuation (26 o
	12	845	40.2	861	2	DD097361 CORYNEBAC
	13	845	40.2	861	2	DD097362 CORYNEBAC
	14	845	40.2	861	2	AX063819 Sequence
	15	845	40.2	861	2	AX063821 Sequence
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c	17	415.6	19.8	453	2	AX122808 Sequence
	18	371.1	17.7	1371	14	DQ019448 Micrococc
	19	371.1	17.7	1380	2	E17152 Micrococcus
	20	303.4	14.4	1395	4	AY702086 Aspergill
	21	303.4	14.4	110000	4	AP007175_15 Continuation (16 o
c	22	296.7	14.1	110000	14	CP000431_73 Continuation (74 o
	23	288.8	13.8	110000	14	CP000656_24 Continuation (25 o
	24	288.5	13.7	110000	14	CP000781_17 Continuation (18 o
c	25	282.5	13.5	110000	14	BA000040_53 Continuation (54 o
c	26	277.6	13.2	110000	14	BA000040_43 Continuation (44 o
c	27	274	13.0	110000	14	CP000454_44 Continuation (45 o
c	28	263.4	12.5	110000	14	CU234118_33 Continuation (34 o
	29	249.1	11.9	110000	14	CP000781_13 Continuation (14 o
	30	248.9	11.9	1368	2	AR319163 AR319163 Sequence

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c	31	245.5	11.7	110000	14	CP000494_37	Continuation (38 o
	32	244.1	11.6	110000	14	CP000521_11	Continuation (12 o
	33	234.1	11.1	110000	14	CR543861_10	Continuation (11 o
	34	220	10.5	110000	14	CP000473_005	Continuation (6 o
	35	209.2	10.0	110000	14	CP000474_03	Continuation (4 o
c	36	205.9	9.8	110000	14	CP000325_38	Continuation (39 o
	37	203.4	9.7	110000	14	AM711867_00	AM711867 Clavibact
	38	196.2	9.3	110000	14	CP000449_31	Continuation (32 o
	39	194.8	9.3	110000	14	CP000353_01	Continuation (12 o
	40	188.3	9.0	110000	14	CP000117_11	Continuation (12 o
c	41	178.6	8.5	110000	14	CP000473_067	Continuation (68 o
c	42	175.5	8.4	110000	14	BA000019_35	Continuation (36 o
	43	172.8	8.2	110000	14	CT573326_35	Continuation (36 o
c	44	172.1	8.2	110000	14	BX571966_08	Continuation (9 o
c	45	172.1	8.2	110000	14	CP000011_08	Continuation (9 o

RESULT 3
BA000036 26/c
WFOCOMMENT

Sequence split into 33 fragments LOCUS BA000036 Accession BA000036

Fragment Name	Begin	End
BA000036_00	1	110000
BA000036_01	100001	210000
BA000036_02	200001	310000
BA000036_03	300001	410000
BA000036_04	400001	510000
BA000036_05	500001	610000
BA000036_06	600001	710000
BA000036_07	700001	810000
BA000036_08	800001	910000
BA000036_09	900001	1010000
BA000036_10	1000001	1110000
BA000036_11	1100001	1210000
BA000036_12	1200001	1310000
BA000036_13	1300001	1410000
BA000036_14	1400001	1510000
BA000036_15	1500001	1610000
BA000036_16	1600001	1710000
BA000036_17	1700001	1810000
BA000036_18	1800001	1910000
BA000036_19	1900001	2010000
BA000036_20	2000001	2110000
BA000036_21	2100001	2210000
BA000036_22	2200001	2310000
BA000036_23	2300001	2410000
BA000036_24	2400001	2510000
BA000036_25	2500001	2610000
BA000036_26	2600001	2710000
BA000036_27	2700001	2810000
BA000036_28	2800001	2910000
BA000036_29	2900001	3010000
BA000036_30	3000001	3110000
BA000036_31	3100001	3210000
BA000036_32	3200001	3309401

Continuation (27 of 33) of BA000036 from base 2600001 (BA000036 *Corynebacterium glutamicum* ATCC 13032 DNA, complete genome. 5/2007)

Query Match 96.1%; Score 2018.2; DB 14; Length 110000;
Best Local Similarity 97.7%; Pred. No. 0;
Matches 2067; Conservative 0; Mismatches 28; Indels 20; Gaps 3;

Qy	1	CACAAAATCCGSGGAATCCACCGAAATCGCTTTCATCTTTGGCTTGATCAAAATGCTTCAT	60
Db	28499	CACAAAATCCGSGGAATCCACCGAAATCGCTTTCATCTTTGGCTTGATCAAAATGCTTCAT	28440
Qy	61	TCGGCCCGGCTGCACCGTCACGCTTCGAGAAATAGAAATAGCGCTTCGACCGCACCC	120
Db	28439	TCGGCCCGGCTGCACCGTCACGCTTCGAGAAATAGAAATAGCGCTTCGACCGCACCC	28382

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QY 121 ACTCTCAACGGCAGCC-----GCCAGCGCGTGGCATCAGCCAGGATTAT 166
 Db 28381 ---CTCAACGGCAGCGCCAGCGAGCCTGTGCCAGCGCGTGGCATCAGCCAGGATTAT 28325

QY 167 TAGGACCGGCGATATAGGTAATGGAGTGGCACCCCTGATCCACCAAAATGCACACAGCCT 226
 Db 28324 TAGGACCGGCGATATAGGTAATGGAGGCGCACCCCTGATCCACCAAAATGCACACAGCCT 28265

QY 227 TCGCGGTACCGTCGTAGTTATCCACCATCAGCGTGGGAAATACCTTGCACCTTCACGGCTCA 286
 Db 28264 TCGGCGCACCGTCGTAGTTATCCACCATCAGCGTGGGAAATACCTTGCACCTTCACGGCTCA 28205

QY 287 TTAATACAGTGGGAAATTTCCCGCGCGACTTTGTGGATCTCACCAGAAATCCATCCTTGAAG 346
 Db 28204 TTAATACAGTGGGAAATTTCCCGCGCGACTTTGTGGATCTCACCAGAAATCCATCCTTGAAG 28145

QY 347 CAGCGAGCAATAAGGCATCGCGCTGGGGAGCATCTTGTCCAGCACCTCCCTGGACTTAA 406
 Db 28144 CAGCGAGCAATAAGGCATCGCGCTGGGGAGCATCTTGTCCAGCACCTCCCTGGACTTAA 28085

QY 407 TCGCGCACTCCCGGGCGTCGACACGGCGAACCGTATAGCCCTGAGTGCTTGGGGCATGCT 466
 Db 28084 TCGCGCACTCCCGGGCGTCGACACGGCGAACCGTATAGCCCTGAGTGCTTGGGGCATGCT 28025

QY 467 GCGCGCCCTGGAAAAATTTCCAAGAAAGGGATTTCGATGCATCGGTGGCAACCATACGGA 526
 Db 28024 GCGCGCCCGGAAAAATTTCCAAGAAAGGGATTTCGATGCATCGGTGGCGCAACCATACGGA 27965

QY 527 TGATACCGGTGTTTTGGCGGTGAAAAGCCTGAGTTTCCACACGCGTTGGGGATTTTCTCC 586
 Db 27964 TGAGCCCGGTGTTTTGGCGGTGAAAAGCCTGAGTTTCCACACGCGTTGGGGATTTTCTCC 27905

QY 587 CGAGTGGAAAACTCACTCGCCGAGGCTGCGAAAAACCGCGCGACACAGTGGAGGGGAG 646
 Db 27904 CGAGTGGAAAACTCACTCGCCGAGGCTGCGAAAAACCGCGCGACACAGTGGAGGGGAG 27845

QY 647 ACGCCAGCGACTTTTGGGACATCATAAATGGTGGCTTTTGAGTCGCTGTG-GCCCCAGAA 705
 Db 27844 ACGCCAGCGACTTTTGGGACATCATAAATGGTGGCTTTTGAGTCGCTGTGAGCCCAAGAA 27785

QY 706 TCTGTCATGCACAAGAGTATATAGCGCAAAAGAAATCACTAGTCTTGATTCTATGTTGAC 765
 Db 27784 TCTGTCATGCACAAGAGTATATAGCGCAAAAGAAATCACTAGTCTTGATTCTATGTTGAC 27725

QY 766 GATGCGGATACCCGAGTACCTGCACGAAATTTTAGATGATGTCCGCGACACCACTCCGG 825
 Db 27724 GATGCGGATACCCGAGTACCTGCACGAAATTTTAGATGATGTCCGCGACACCACTCCGG 27665

QY 826 CSAGTTGGCGGATTACATCCCGGAACTAATAATCTGCGGACCCAAACCCGCTGCGAGTAGC 885
 Db 27664 CSAGTTGGCGGATTACATCCCGGAACTAATAATCTGCGGACCCAAACCCGCTGCGAGTAGC 27605

QY 886 CCTGTGCAACCGTTAACGGACACATCTACAGCGCAGGGGATGACGACATCGAATTCACCAT 945
 Db 27604 CCTGTGCAACCGTTAACGGACACATCTACAGCGCAGGGGATGACGACATCGAATTCACCAT 27545

QY 946 GCAAAGTATTTCCAAGCCCTTTTGCTTACGCACTCGCACTCCAAGAAATCGGGCTTTGATGA 1005
 Db 27544 GCAAAGTATTTCCAAGCCATTTGCTTACGCACTCGCACTCCAAGAAATCGGGCTTTGATGA 27485

QY 1006 GGTCTCTGCATCCGTGCGCTTGGAAACCCCTCCGCTGAGGCGCTTCAACGAAATTTCCCTCGA 1065
 Db 27484 GGTCTCTGCATCCGTGCGCTTGGAGCCCTCCGCTGAGGCGCTTCAACGAAATTTCCCTCGA 27425

QY 1066 CGCGCAAAACCGGCCCATGAACCCCATGATCAACCGCGGCGGATCGGCATCAACAGCT 1125
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QY 1126 GATCAACGGCTCCGACTCCACCGCTGGAAAGACCGCTGGAAAAATCCGACACTACTTCTC 1185
 Db 27364 GATCAACGGCTCCGATTCCACCGCTGGAAAGACCGCTGGAAAAATCCGACACTACTTCTC 27305

QY 1186 TGAACCTTGTGGACGCGAACTCACCATCGACCGCGTGTCTGCCGAATCCGAACTCGCGGG 1245
 Db 27304 TGAACCTTGTGGACGCGAACTCACCATCGACCGCGTGTCTGCCGAATCCGAACTCGCGGG 27245

QY 1246 CGCCGACCGCAACCTCTCCATCGGCCACATGCTGCGCAACTATGGCGTCATCGAAAGCA 1305
 Db 27244 CGCCGACCGCAACCTCTCCATCGGCCACATGCTGCGCAATTACGGCGTCATCGAAAGCA 27185

QY 1306 AGCCCAAGACGCGCTCTCCAGCTACACGCTGCAATGTGCCATCAAAGTAACACGCGCA 1365
 Db 27184 AGCCCAAGACGCGCTCTCCAGCTACACGCTGCAATGTGCCATCAAAGTAACACGCGCA 27125

QY 1366 CCTCGCAGTCATGACGCGCACGCTGCGCGCGCGGCGAGCACCCTAATTACCGGCAAGAA 1425
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QY 1426 GCTTCTCGACGCGCGCGCTGTGCGCGCTCACCTCTCGGTCTCAGGAGGATGTA 1485
 Db 27064 GCTTCTCGACGCGCGCGCTGTGCGCGCTCACCTCTCGGTCTCAGGAGGATGTA 27005

QY 1486 CGACGAGGACGCGCAGTGGCTCTCCACCGTAGGCATCCCGCGAAAATCAGGAGTCGCGG 1545
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QY 1546 CGGACTCATCGGCATTGTGCCAGGTGACGTGGGCATCGGCACATTTTCCCGACGCGTGAA 1605
 Db 26944 CGGACTCATCGGCATTGTGCCAGGTGACGTGGGCATCGGCACATTTTCCCGACGCGTGAA 26885

QY 1606 CCCCAAGGCAACAGCGCTGCGCGCGGTAAAAATATTCAAACAGCTTTCCGACGACATGGG 1665
 Db 26884 CCCCAAGGCAACAGCGCTGCGCGCGGTAAAAATATTCAAACAGCTTTCCGACGACATGGG 26825

QY 1666 CTTCCACCTTATGTCCACCGAGCAGGTATCCGSGCCAGCGATACGATCCATTACGCGGGA 1725
 Db 26824 CTTCCACCTTATGTCCACCGAGCAGGTATCCGSGCCAGCGATACGATCCATTACGCGGGA 26765

QY 1726 CGGCGACACCACTTCATCCAAATGCAGGCGGCCATGAACCTTCAGCGACGCAAGCCT 1785
 Db 26764 CGGCGACACCACTTCATCCAAATGCAGGCGGCCATGAACCTTCAGCGACGCAAGCCT 26705

QY 1786 CTTCCACGCCATCGTGGAAACACAACCTTTGAAGGCACCGAAGTTGTTCTTGATCTCACCG 1845
 Db 26704 CTTCCACGCCATCGTGGAAACACAACCTTTGAAGGCACCGAAGTTGTTCTTGATCTCACCG 26645

QY 1846 AGTACTTAGCTTCCACCCGTAGCCATCGGCATGATCAAAGAAAGGCTCAAAGCATCCG 1905
 Db 26644 AGTACTTAGCTTCCACCCGTAGCCATCGGCATGATCAAAGAAAGGCTCAAAGCATCCG 26585

QY 1906 CGACGACGCGCTTTGAGGTGTTTCATCCTCGACCCAGATGACGTACTGCGCGAATTTCATGTT 1965
 Db 26584 CGACGACGCGCTTTGAGGTGTTTCATCCTCGACCCAGATGACGTACTGCGCGAATTTCATGTT 26525

QY 1966 TTCCGACGCGACCATCTGCAAGAAACGAGTGTGACCGGTAGCTTTATGGTCTGAACAAIT 2025
 Db 26524 TTCCGACGCGACCATCTGCAAGAAACGAGTGTGACCGGTAGCTTTATGGGCTGAACAAIT 26465

QY 2026 CGAAGGAGATTAAATCGGTGAAAAAGAAAGCTTATGTTGCCCTTGATTGTTGACGCTTTGGG 2085
 Db 26464 CTAAGGAGAAATTATCGGTGAAAAAGAAAGCTTATGTTGCCCTTGATTGTTGACGCTTTGGG 26405

QY 2086 ATTAAGTGCTGCG 2100
 Db 26404 GTTAAGTGCTGCG 26390

Art Unit: 1652

GenCore version 6.2.1

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OM nucleic - nucleic search, using sw model

Run on: August 6, 2008, 16:09:03 ; Search time 72451 Seconds
 (without alignments)
 2372.720 Million cell updates/sec

Title: US-10-720-177-1
 Perfect score: 2100
 Sequence: 1 cacaaaatcggcggaatcca.....ttgggataagtgcctgcag 2100

Scoring table: **IDENTITY NUCXX**
Gapop 10.0 , Gapext 1.0 <<<<<<<

Searched: 9588671 seqs, 40929980300 residues

Total number of hits satisfying chosen parameters: 19177342

Minimum DB seq length: 0

Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
 Maximum Match 100%
 Listing first 45 summaries

Database : GenEmbl:*
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 2: gb_pat:*
 3: gb_ph:*
 4: gb_pl:*
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 6: gb_ro:*
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 12: gb_in:*
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 14: gb_ba:*
 15: gb_htgl:*
 16: gb_htg2:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result	No.	Score	% Query Match	Length	DB	ID	Description
	1	2100	100.0	2100	2	CQ817719	CQ817719 Sequence
	2	2100	100.0	2100	2	DD028154	DD028154 Method fo
c	3	2004.4	95.4	110000	14	BA000036_26	Continuation (27 o <<<<<
c	4	2004.4	95.4	349136	14	BX927155	BX927155 Corynebac
c	5	2004.4	95.4	349980	2	AXI27151	AXI27151 Sequence
c	6	1924.8	91.7	110000	14	AP009044_26	Continuation (27 o
	7	1588.2	75.6	1629	2	BD164926	BD164926 Novel pol
	8	1588.2	75.6	1629	2	AXI22809	AXI22809 Sequence
	9	1435.2	68.3	1468	2	AX764345	AX764345 Sequence
	10	1435.2	68.3	1468	2	EA032755	EA032755 Sequence

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13	845	40.2	861	2	AX063819	AX063819 Sequence
14	845	40.2	861	2	AX063821	AX063821 Sequence
c 15	669.4	31.9	110000	14	BA0000035_25	Continuation (26 o
c 16	415.6	19.8	453	2	BD164925	BD164925 Novel pol
c 17	415.6	19.8	453	2	AX122808	AX122808 Sequence
18	365.4	17.4	1371	14	DQ019448	DQ019448 Micrococc
19	365.4	17.4	1380	2	E17152	E17152 Micrococcus
20	296.8	14.1	1395	4	AY702086	AY702086 Aspergill
21	296.8	14.1	110000	4	AP007175_15	Continuation (16 o
c 22	279.2	13.3	110000	14	CP000781_17	Continuation (18 o
23	275.6	13.1	110000	14	CP000656_24	Continuation (25 o
c 24	275	13.1	110000	14	CP000431_73	Continuation (74 o
c 25	273	13.0	110000	14	BA0000040_53	Continuation (54 o
c 26	272.2	13.0	110000	14	BA0000040_43	Continuation (44 o
c 27	258	12.3	110000	14	CU234118_33	Continuation (34 o
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29	241	11.5	110000	14	CP000781_13	Continuation (14 o
c 30	240	11.4	110000	14	CP000494_37	Continuation (38 o
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34	216.8	10.3	110000	14	CP000473_005	Continuation (6 of
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c 36	194.2	9.2	110000	14	CP000325_38	Continuation (39 o
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c 41	167.8	8.0	110000	14	CP000473_067	Continuation (68 o
c 42	167.4	8.0	110000	14	BA0000019_35	Continuation (36 o
43	164.2	7.8	110000	14	AE014292_03	Continuation (4 of
c 44	163.4	7.8	110000	14	BX571966_08	Continuation (9 of
c 45	163.4	7.8	110000	14	CP000011_08	Continuation (9 of

RESULT 3

BA000036_26/c

WPCOMMENT

Sequence split into 33 fragments LOCUS BA000036 Accession BA000036

Fragment Name	Begin	End
BA000036_00	1	110000
BA000036_01	100001	210000
BA000036_02	200001	310000
BA000036_03	300001	410000
BA000036_04	400001	510000
BA000036_05	500001	610000
BA000036_06	600001	710000
BA000036_07	700001	810000
BA000036_08	800001	910000
BA000036_09	900001	1010000
BA000036_10	1000001	1110000
BA000036_11	1100001	1210000
BA000036_12	1200001	1310000
BA000036_13	1300001	1410000
BA000036_14	1400001	1510000
BA000036_15	1500001	1610000
BA000036_16	1600001	1710000
BA000036_17	1700001	1810000
BA000036_18	1800001	1910000
BA000036_19	1900001	2010000
BA000036_20	2000001	2110000
BA000036_21	2100001	2210000
BA000036_22	2200001	2310000
BA000036_23	2300001	2410000
BA000036_24	2400001	2510000
BA000036_25	2500001	2610000
BA000036_26	2600001	2710000

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BA000036.27 2700001 2810000
 BA000036.28 2800001 2910000
 BA000036.29 2900001 3010000
 BA000036.30 3000001 3110000
 BA000036.31 3100001 3210000
 BA000036.32 3200001 3309401

Continuation (27 of 33) of BA000036 from base 2600001 (BA000036 *Corynebacterium glutamicum* ATCC 13032 DNA, complete genome. 5/2007)

Query Match		95.4%;		Score 2004.4;		DB 14;		Length 110000;	
Best Local Similarity		97.6%;		Pred. No. 0;					
Matches 2059;		Conservative		0;		Mismatches		41;	
						Indels		10;	
								Gaps	
								2;	
Qy	1	CACAAAATCCGCGAATCCACCGAAATCGCTTTCATCTTTGGCTTGATCAAATGCTCAT	60						
Db	28499	CACAAAATCCGCGAATCCACCGAAATCGCTTTCATCTTTGGCTTGATCAAATGCTCAT	28440						
Qy	61	TCGGCCCGGCTGCACCGTCACGCTTCGAGAAATAGAAATAGCGCTTGTGAGCCACCC-	119						
Db	28439	TCGGCCCGGCTGCACCGTCACGCTTCGAGAAATAGAAATAGCGCTTGTGAGCCACCC	28380						
Qy	120	-----CACTCTCAACGCGACGCGCCAGCGGTGGCATCAGCCAGGATTATTAGGA	171						
Db	28379	CAACGCGACGCGCCAGCGGCTTGTGCAAGCGGTGGCATCAGCCAGGATTATTAGGA	28320						
Qy	172	CCGGCGATATAGTAAATGGAGTGGCACCCCTGATCCACAAATGCACCAAGCCTTCGCC	231						
Db	28319	CCGGCGATATAGTAAATGGAGCGGCAACCCCTGATCCACAAATGCACCAAGCCTTCGCC	28260						
Qy	232	GTACCGTGTAGTTATCCACCATCAGCTGGGAATACCTTGCACTTCAGGCTCAATTA	291						
Db	28259	GCACCGTGTAGTTATCCACCATCAGCTGGGAATACCTTGCACTTCAGGCTCAATTA	28200						
Qy	292	ACAGTGGGAATTTCCGCGCGACTTTTGTGATCTCACCAGATCCATCTTGAAGCAGCG	351						
Db	28199	ACAGTGGGAATTTCCGCGCGACTTTTGTGATCTCACCAGATCCATCTTGAAGCAGCG	28140						
Qy	352	AGCAATAAGCCATCGGGGTGGGGGACGATCTTGTCCAGCACTCCCTTGGACTTAATCGCC	411						
Db	28139	AGCAATAAGCCATCGGGGTGGGGGACGATCTTGTCCAGCACTCCCTTGGACTTAATCGCC	28080						
Qy	412	GACTCCCGGGCGTCGACAAAGCGCAACCGTATAGCCCTGAGTGTCTGCGGCACTGTCGCG	471						
Db	28079	GACTCCCGGGCGTCGACAAAGCGCAACCGTATAGCCCTGAGTGTCTGCGGCGCTGTCGCG	28020						
Qy	472	CCCTGGAAAAATTTCCAAAGAAAGGATTTCGATGCATCGGTGGCAACCATAGCAGTATA	531						
Db	28019	CCCGGAAAAATTTCCAAAGAAAGGATTTCGACGATCGCGGCAACCATAGCAGTATAGG	27960						
Qy	532	CCGCTGTTTTTGGCGCTGAAAAGCCTGAGTTTCCACACGCGTTGCGGATTTTTCGCGAGT	591						
Db	27959	CCGCTGTTTTTGGCGCTGAAAAGCCTGAGTTTCCACACGCGTTGCGGATTTTTCGCGAGT	27900						
Qy	592	GGAAAACTCACTCGGCCAGGCTGCGAAAACGCCGCCGACACAGTGGGAAGGGAGACGCC	651						
Db	27899	GGAAAACTCACTCGGCCAGGCTGCGAAAACGCCGCCGACACAGTGGGAAGGGAGACGCC	27840						
Qy	652	AGCGACTTTTTCGCGACATATAAATGGTGGCTTTTGAAGTGCCTGTG-GCCCCAGAACTGT	710						
Db	27839	AGCGACTTTTTCGCGACATATAAATGGTGGCTTTTGAAGTGCCTGTGAGCCCCAGAACTGT	27780						
Qy	711	CATGCACAAGAGTATATAGCGCAAAAGAAATCACTAGTCTTGTATCTATGTGACGATGC	770						
Db	27779	CATGCACAAGAGTATATAGCGCAAAAGAAATCACTAGTCTTGTATCTATGTGACGATGC	27720						
Qy	771	CGATACCGGAGTACCTGCACGAAATTTAGATGATCTCGCGACACCACTCCGGCGAGT	830						
Db	27719	CGATACCGGAGTACCTGCACGAAATTTAGATGATCTCGCGACACCACTCCGGCGAGT	27660						

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Qy	831	TGSCCGATTACATCCCGGAACATAAAATCTGCCGACCCAAACCGGTGGCAGTAGCCCTGT	890
Db	27659	TGSCCGATTACATCCCGGAACATAAAATCTGCCGACCCAAACCGGTGGCAGTAGCCCTGT	27600
Qy	891	GCACCGTTAAGCGACACATCTACAGCGCAGGCGGATGACGACATCGAATTACACATGCAAA	950
Db	27599	GCACCGTTAAGCGACACATCTACAGCGCAGGCGGATGACGACATCGAATTACACATGCAAA	27540
Qy	951	GTATTTCCAAGCCCTTTGCGCTACGCACTCGCACTCCAAGAAATCGGCTTTGATGAGGTCT	1010
Db	27539	GTATTTCCAAGCCATTTGCGCTACGCACTCGCACTCCAAGAAATCGGCTTTGATGAGGTCT	27480
Qy	1011	CTGCATCCGTTGCGCTTGGAAACCTCCGGTGAGGCTTCAACGAACCTTCCCTCGACGGCG	1070
Db	27479	CTGCATCCGTTGCGCTTGGAAACCTCCGGTGAGGCTTCAACGAACCTTCCCTCGACGGCG	27420
Qy	1071	AAAACGCGCCCATGAACCCCATGATCAACGCGCGCGGATCGGCATCAACAGCTGATCA	1130
Db	27419	AAAACGCGCCCATGAACCCCATGATCAACGCGCGCGGATCGGCATCAACAGCTGATCA	27360
Qy	1131	ACGGCTCGGACTCCACCGTGGAAAGACCGAGTGGAAAAAATCCGACACTACTTCTCTGAAC	1190
Db	27359	ACGGCTCGGATTCCACCGTGGAAAGACCGGTTGAAAAAATCCGACACTACTTCTCTGAAC	27300
Qy	1191	TTGCTGAGCGGAACTGACCATCGACCGGTGCTTGCGGAATCGGAATCGCGCGCGCG	1250
Db	27299	TTGCTGAGCGGAACTGACCATCGACCGGTGCTTGCGGAATCGGAATCGCGCGCGCG	27240
Qy	1251	ACCGCAACCTCTCCATCGGCCCATGCTGCGCAACTATGGGCTCATCGAAGACGAAGCC	1310
Db	27239	ACCGCAACCTCTCCATCGGCCCATGCTGCGCAATTACGGGCTCATCGAAGACGAAGCC	27180
Qy	1311	ACGACGCGTCTCTCAGCTACAGCGTGAATGTGCCATCAAAGTAACACGCGGCAACCTCG	1370
Db	27179	ACGACGCGTCTCTCAGCTACAGCGTGAATGTGCCATCAAAGTAACACGCGGCAACCTCG	27120
Qy	1371	CAGTCATGACGCGCACGCTCGCGCGCGGCGGCACGACCCCAATTACGGCAAGAAGCTTC	1430
Db	27119	CAGTCATGACGCGCACGCTCGCGCGCGGCGGCACACCCCAATTACGGCAAGAAGCTTC	27060
Qy	1431	TCGACGCGCGGCTCTGCGCGCTCACCTCTCCGTCATGGCTTCAGCAGGCATGTACGACG	1490
Db	27059	TCGACGCGCGGCTCTGCGCGCTCACCTCTCCGTCATGGCTTCAGCAGGCATGTACGACG	27000
Qy	1491	AGGCAGGCGAGTGGCTCTCCACCGTAGGCATCCCCGGAAATCAGGAGTCGCGCGGGAC	1550
Db	26999	AGGCAGGCGAGTGGCTCTCCACCGTAGGCATCCCCGGAAATCAGGAGTCGCGCGGGAC	26940
Qy	1551	TCATCGGCATTCTGCGAGTCAGCTGGGCATCGCCACATTTTCCCAAGCCTGAACCCCA	1610
Db	26939	TCATCGGCATTCTGCGAGTCAGCTGGGCATCGCCACATTTTCCCAAGCCTGAACCCCA	26880
Qy	1611	AAGGCACACGCGTGGCGGCGGTAAAAATATTCAAACAGCTTTCGACGACATGGGCTCC	1670
Db	26879	AAGGCACACGCGTGGCGGCGGTAAAAATATTCAAACAGCTTTCGACGACATGGGCTCC	26820
Qy	1671	ACCTTATGTCCACCGAGCAGGTATCCGGCCACGCAAGTACGATCCATTACGCGGGACGGCG	1730
Db	26819	ACCTTATGTCCACCGAGCAGGTATCCGGCCACGCAAGTACGATCCATTACGCGGGACGGCG	26760
Qy	1731	ACACACACCTTCATCCAAATGCAAGGCGCGCATGAACCTTCTCAGCCAGCGAAGCTTCTCTC	1790
Db	26759	ACACACACCTTCATCCAAATGCAAGGCGCGCATGAACCTTCTCAGCCAGCGAAGCTTCTCTC	26700
Qy	1791	ACGCCATCGTGGAAACACAACCTTTGAAGGCAACGAAGTTGTCTTGATCTCAACCGAGTAC	1850
Db	26699	ACGCCATCGTGGAAACACAACCTTTGAAGGCAACGAAGTTGTCTTGATCTCAACCGAGTAC	26640

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Qy      1851  TTAGTTTCACCCCGTAGCCATCCCGATGATCAAAAGAAAGGCGCTCAAACGGATCCGCGACG 1910
      |||
Db      26639  TTAGTTTCACCCCGTAGCCATCCCGATGATCAAAAGAAAGGCGCTCAAACGGATCCGCGACG 26580
      |||
Qy      1911  CAGCGTTTGAGGTGTTGATCCTCGACCCAGATGACGTACTGCCCCGATTTCATGTTTTCCG 1970
      |||
Db      26579  CAGCGTTTGAGGTGTTGATCCTCGACCCAGATGACGTACTGCCCCGATTTCATGTTTTCCG 26520
      |||
Qy      1971  ACGGCACCATCTGC AAAAGAAAGAGTGTGACCGGTAGCTTTATGGCTGTGAACAATTGGAAG 2030
      |||
Db      26519  ACGGCACCATCTGC AAAAGAAAGAGTGTGACCGGTAGCTTTATGGCTGTGAACAATTGTAAG 26460
      |||
Qy      2031  GAGATTAAATCGGTGAAAAAGAGCGCTTATGTTGCCTTTTGATTGTTGCAGCTTTGGGATTAA 2090
      |||
Db      26459  GAGAAATTATCGGTGAAAAAGAGCGCTTATGTTGCCTTTTGATTGTTGCAGCTTTGGGATTAA 26400
      |||
Qy      2091  GTGCCTGCAG 2100
      |||
Db      26399  GTGCCTGCAG 26390

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If the claims were to recite “96% or more homologous to SEQ ID NO: 1”, would the polynucleotide of GenBank accession number BA000036_26 meet the limitation of “96% or more”? The answer to that question is unclear because it depends on how the calculation is made. In the instant case, the claims include a numerical limitation without stating which of the multiple methods of measuring that number should be used. In view of the fact that one of skill in the art cannot reasonably determine which species are encompassed by the claims, the term is deemed indefinite.

Claim Rejections - 35 USC § 112, first paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 4-5, 12-16, 18-19 and 21 remain rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

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Claims 1, 4-5, 12-16, 18-19 and 21 are directed in part to a coryneform bacterium being modified by making any type of mutation to a glutaminase gene on the bacterium's chromosome such that the glutaminase activity in said bacterium (1) is reduced to 0.1/0.01 U/mg cellular protein, or (2) is reduced to 0.1/0.01 U/mg cellular protein and is $\frac{1}{2}$ or less than the glutamine synthetase activity in said bacterium, wherein the glutaminase gene prior the mutation comprises SEQ ID NO: 1, or is a structural variant of the polynucleotide of SEQ ID NO: 1.

The claims require a precise reduction in glutaminase activity to a specific level of glutaminase activity (0.1 U/mg or 0.01U/mg) and a precise ratio of glutamine synthetase activity to glutaminase activity (2:1) obtained by any means. While one could (1) disrupt a gene by introducing deletions/insertions within the coding region of a gene, (2) disrupt the expression of a gene by deleting the regulatory region of a gene such that the protein encoded by that gene is either completely inactive (0 Units/mg) or has some residual activity, (3) enhance the activity of an enzyme by increasing the copy number of the gene encoding said enzyme, or (4) enhance the activity of an enzyme by placing the gene encoding said enzyme under the control of a strong heterologous promoter such that one could increase the amount of that enzyme with respect to other proteins, neither the specification nor the art provide adequate description of the genus of methods by which one could achieve the required activity levels. It is noted that the claims require a precise level of reduction in enzymatic activity and/or a precise ratio of enzymatic activities. As such, the specification should provide some description as to how one of skill in the art should mutate the recited glutaminase genes such that those mutations would result in that precise level of enzymatic activity reduction in any coryneform bacterium as recited, or how should one modulate expression of the recited genes to obtain the desired glutaminase activity. Similarly, in view of the fact that the claims require a precise ratio of glutamine synthetase to glutaminase activity, the specification should provide adequate description as to the modifications that can be made to a coryneform bacterium as recited such that one could achieve the required ratio. In addition, it should be noted some of the

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claims are not limited to a particular glutamine synthetase activity. Therefore, these claims encompass a coryneform bacterium where a gene encoding glutamine synthetase activity from any organism is modified in any way to modulate glutamine synthetase activity such that the recited ratio is achieved.

Neither the specification nor the art provide a structure/function correlation for glutaminases or glutamine synthetases that would allow one of skill in the art to envision the structural modifications required in the recited genes to obtain the desired reduction in glutaminase activity, or the desired ratio of glutaminase to glutamine synthetase activity. The art as previously discussed clearly teaches the unpredictability of determining *a priori* the effect of structural changes on a protein's function based solely on structural similarity. For example, Witkowski et al. teach that even a single conservative substitution can result in enzymatic activity changes. Seffernick et al. (J. Bacteriol. 183(8):2405-2410, 2001) teach that two naturally occurring *Pseudomonas* enzymes having 98% sequence identity catalyze two different reactions: deamination and dehalogenation, therefore having different functions. Therefore, in view of the lack of information as to a structure/function correlation or some guidance as to which are the mutations required to obtain the desired reduction in glutaminase activity and/or the desired ratio of glutaminase to glutamine synthetase activity, one of skill in the art cannot reasonably conclude that the claimed invention is adequately described by the teachings of the specification.

Claims 1, 4-5, 12-16, 18-21 remain rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a *C. glutamicum* cell, wherein said cell has been modified to reduce the endogenous glutaminase activity and to increase glutamine synthetase activity, wherein the reduction in glutaminase activity is due to an inactivating deletion in the endogenous glutaminase gene of said *C. glutamicum* cell, wherein the endogenous glutaminase gene comprises SEQ ID NO: 1 prior to the introduction of the deletion, and the increase in glutamine synthase activity is due to (i) an increase in the copy number of the *C. glutamicum* glnA gene of SEQ ID NO: 3, or (ii) an increase in expression of the

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C. glutamicum glnA gene of SEQ ID NO: 3 by placing said gene under the control of a heterologous promoter, does not reasonably provide enablement for (1) a coryneform bacterium modified to reduce glutaminase activity to less than 0.1 U/mg protein or 0.01 U/mg protein in said bacterium by mutating any region of a glutaminase gene comprising SEQ ID NO: 1 or a structural homolog thereof, (2) the coryneform bacterium of (1) further modified in any way to modulate any glutamine synthetase activity such that the recited ratio (2 to 1) of glutamine synthetase activity to glutaminase activity is achieved, or (3) the coryneform bacterium of (1) further modified by increasing the expression of a glutamine synthetase gene which is a structural homolog of the nucleic acid of SEQ ID NO: 3, wherein said increase in expression is obtained by increasing the copy number of the glutamine synthetase gene or by replacing the endogenous promoter of the glutamine synthetase gene with a stronger promoter. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2d 1400 (Fed. Cir. 1988)) as follows: (1) quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence and absence of working examples, (4) the nature of the invention, (5) the state of prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. The factors which have lead the Examiner to conclude that the specification fails to teach how to make and/or use the claimed invention without undue experimentation, are addressed in detail below.

The breadth of the claims. Claims 1, 4-5, 12-16, 18-21 are so broad as to encompass (1) a coryneform bacterium modified by making any type of mutation to a glutaminase gene to reduce glutaminase activity to less than 0.1 U/mg protein or 0.01 U/mg protein in said bacterium, wherein said glutaminase gene prior to being mutated either comprises SEQ ID NO: 1 or is a structural variant of the polynucleotide of SEQ ID NO: 1, (2) the coryneform bacterium of (1) further modified in any way to

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modulate any glutamine synthetase activity such that the recited ratio (2 to 1) of glutamine synthetase activity to glutaminase activity is achieved, or (3) the coryneform bacterium of (1) further modified by increasing the expression of a glutamine synthetase gene which is a structural variant of the nucleic acid of SEQ ID NO: 3, wherein said increase in expression is obtained by increasing the copy number of the glutamine synthetase gene or by replacing the endogenous promoter of the glutamine synthetase gene with a stronger promoter. It is noted that some of the claims, such as claims 4 and 15, do not structurally limit the glutamine synthetase gene, thus the claimed bacteria can express any gene encoding any glutamine synthetase.

The enablement provided is not commensurate in scope with the claims due to the lack of information as to (A) the mutations which would result in (1) a coryneform bacterium to have a glutaminase activity which is 0.1/0.01 U/mg cellular protein or less, or (2) a coryneform bacterium to have the recited glutaminase activity and the recited ratio of glutaminase to glutamine synthetase activity, (B) the structure of any glutamine synthetase gene and mutations required to modulate the expression of said gene so that the recited ratio of glutaminase to glutamine synthetase is obtained, and (C) the structural features required in any structural variant of the polynucleotide of SEQ ID NO: 3 such that it can encode a protein having glutamine synthetase activity. In the instant case, the specification enables a *C. glutamicum* cell, wherein said cell has been modified to reduce the endogenous glutaminase activity and to increase glutamine synthetase activity, wherein the reduction in glutaminase activity is due to an inactivating deletion in the endogenous glutaminase gene of said *C. glutamicum* cell, wherein the endogenous glutaminase gene comprises SEQ ID NO: 1 prior to the introduction of the deletion, and the increase in glutamine synthase activity is due to (i) an increase in the copy number of the *C. glutamicum* glnA gene of SEQ ID NO: 3, or (ii) an increase in expression of the *C. glutamicum* glnA gene of SEQ ID NO: 3 by placing said gene under the control of a heterologous promoter.

The amount of direction or guidance presented and the existence of working examples. The specification discloses a mutant *C. glutamicum* cell which has an inactivating deletion in the *C. glutamicum* gene comprising SEQ ID NO: 1 (pages 25-27) and has been further transformed with the *C. glutamicum* glnA gene to increase the copy number of the glnA gene of SEQ ID NO: 3 (pages 28-30), as a working example. However, the specification fails to disclose (1) other mutations that can be made to a glutaminase gene to obtain the precise reduction in activity, (2) which modifications can be made to the cell and/or any glutamine synthetase gene to obtain the precise glutaminase to glutamine synthetase ratio, or (3) a structure/function correlation that would provide one of skill in the art with the structural features required in any structural variant of the polynucleotide of SEQ ID NO: 3 to encode a glutamine synthetase.

The state of prior art, the relative skill of those in the art, and the predictability or unpredictability of the art. The nucleotide sequence of the coding region of a polynucleotide encoding a protein determines the structural and functional properties of that protein. In the instant case, neither the specification nor the art provide a correlation between structure and activity such that one of skill in the art can envision the structure of any nucleic acid encoding a glutamine synthetase, or the identifying structural features associated with polynucleotides encoding glutamine synthetases. Furthermore, neither the specification nor the art provide any teaching or guidance as to how the structures of those glutaminases and glutamine synthetases known in the art correlate with those enzymatic activities such that one of skill in the art would know which structural modifications are required to obtain the desired effect in glutaminase and glutamine synthetase activity (i.e., reduction or enhancement). The art clearly teaches that structural changes in a protein to obtain the desired activity without any guidance/knowledge as to which amino acids in a protein are associated with the desired effect/activity is highly unpredictable. For example, Branden et al. (Introduction to Protein Structure, Garland Publishing Inc., New York, page 247, 1991) teach that (1) protein engineers are frequently surprised by the range of effects caused by

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single mutations that they hoped would change only one specific and simple property in enzymes, (2) the often surprising results obtained by experiments where single mutations are made reveal how little is known about the rules of protein stability, and (3) the difficulties in designing *de novo* stable proteins with specific functions. The teachings of Branden et al. are further supported by the teachings of Witkowski et al. and Seffernick et al. already discussed above, where it is shown that even small structural changes result in enzymatic activity changes.

The quantity of experimentation required to practice the claimed invention based on the teachings of the specification. While methods of generating or isolating variants of a polynucleotide were known in the art at the time of the invention, it was not routine in the art to screen by a trial and error process for any number of polynucleotides and determine which ones encode glutamine synthetases. In addition, it was not routine in the art to screen by trial and error for (1) essentially an infinite number of mutations in either the regulatory region of a gene or in the coding region of a gene to determine which ones result in reduced glutaminase activity or enhanced glutamine synthetase activity, as recited in the claims, (2) all possible enhancers of glutamine synthetase activity such as chemicals and the products of other genes, or (3) all possible transcription enhancers of genes encoding glutamine synthetases such as chemicals and the products of other genes. It is noted that claims 4 and 15 do not limit the means by which one can obtain the recited ratio, thus including not only mutations to a gene encoding a glutamine synthetase but also including the use of chemical enhancers/inducers as well as the expression of proteins which would act as transcription/activity enhancers.

As indicated in the action mailed on 3/5/2007, the total number of variants of a polynucleotide having a specific sequence identity can be calculated from the formula $N! \times 3^A / (N-A)! / A!$, where N is the length in nucleotides of the reference polynucleotide and A is the number of allowed substitutions for a specific % identity. Thus, for a variant of the polynucleotide of SEQ ID NO: 3 having, for example, 95% sequence identity to the polynucleotide of SEQ ID NO: 3, the total number of variants to be tested is

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$2500! \times 3^{125} / (2500 - 125)! / 125!$ (SEQ ID NO: 3 has 2500 nucleotides; $125 = 0.05 \times 2500$) or 5.3×10^{273}

variants. While many of these variants are going to be degenerate variants of the polynucleotide of SEQ ID NO: 3 and can be excluded from additional testing, there are some that will not encode the glutamine synthetase of SEQ ID NO: 4 (encoded by the polynucleotide of SEQ ID NO: 3). These variants of the polypeptide of SEQ ID NO: 4 can have up to 74% sequence identity to the polypeptide of SEQ ID NO: 4 in view of the fact that 125 nucleotide mismatches (5% variability in 95% sequence identity variants) can affect 125 codons ($74\% = 100\% - 125 \times 100 / 477$; SEQ ID NO: 4 = 477 amino acids). In the absence of a structure/function correlation or some knowledge or guidance as to which of the variants that do not encode the polypeptide of SEQ ID NO: 4 are more likely to encode a protein having glutamine synthetase activity, one of skill in the art would have to test an essentially infinite number of variants and determine which ones have the desired activity. Similarly, in the absence of some guidance as to the mutations that can be made to a glutaminase or glutamine synthetase gene, and the modifications that can be made to a coryneform bacteria to achieve the precise glutaminase activity and the precise ratio of glutaminase to glutamine synthetase activity, one of skill in the art would have to conduct a large number of tests to determine which modifications/mutations produce the desired result. While enablement is not precluded by the need of routine experimentation, in the instant case, the amount of experimentation required to enable the full scope of the claims is undue.

Therefore, taking into consideration the extremely broad scope of the claims, the lack of guidance, the amount of information provided, the lack of knowledge about a correlation between structure and function, and the high degree of unpredictability of the prior art in regard to structural changes and their effect on function, one of ordinary skill in the art would have to go through the burden of undue experimentation in order to practice the claimed invention. Thus, Appellant has not provided sufficient guidance to enable one of ordinary skill in the art to make and use the invention in a manner reasonably correlated with the scope of the claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1, 4-5, 13-16, 19-21 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Nakamura et al. (EP 1229121 A2 published 8/7/2002; cited in the IDS) in view of Pompejus et al. (WO 01/00843, published 1/4/2001; cited in the IDS), Jakoby et al. (GenBank accession number Y13221, 1997; FEMS Microbiol. Lett. 154(1):81-88, 1997; both cited in the IDS), and further in view of Duran et al. (Microbiology 141:2883-2889, 1995), as evidenced by Nakagawa et al. (GenBank accession number AX127151, 2001; EP 1 108 790 published 6/20/2001).

Nakamura et al. teach a method for producing L-glutamine by fermentation of an L-glutamine producing *C. glutamicum* cell, wherein said cell has been modified to increase the intracellular concentration of glutamine synthetase by increasing the copy number of the *glnA* gene of *C. glutamicum* (encodes glutamine synthetase; Example 1, Table 1, strain AJ12418/pGS). Nakamura et al. also teach a method for production of L-glutamine and suppression of L-glutamic acid as a by-product (paragraph [005]-[006]). Nakamura et al. do not teach a method for producing L-glutamine wherein glutaminase activity is reduced.

Duran et al. teach that glutaminase degrades glutamine to yield glutamate and ammonium (page 2884, left column, first full paragraph) and disclose a mutant *R. etli* (LM16) wherein the chromosomal glutaminase gene is disrupted by Tn5 mutagenesis (Page 2884, Methods, Strains and Plasmid). The reference also teaches that LM16 produces more glutamine than glutamate when cultured with different substrates (page 2886, Table 1). As shown in Table 1, the amount of glutamine produced varies from

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53X (49/0.9) to 2X (0.8/04) more glutamine in the glutaminase deficient mutant LM16 as compared to the wild type *R. etli*. Duran et al. do not teach a *C. glutamicum* or coryneform bacterium deficient in glutaminase.

Jakoby et al. teach a *C. glutamicum* glutamine synthetase gene and its corresponding glutamine synthetase. The gene of Jakoby et al. is 99.1% sequence identical to the polynucleotide of SEQ ID NO: 3 (99.1% = 2479x100/2500). Jakoby et al. do not teach a method for producing L-glutamine in coryneform bacteria wherein glutaminase activity is reduced. For the reader's convenience, a copy of the alignment (provided with the Office action mailed on 10/16/2008) is shown below:

```

RESULT 3
Y13221
LOCUS       Y13221                3686 bp    DNA        linear    BCT 28-AUG-1997
DEFINITION  Corynebacterium glutamicum glnA gene.
ACCESSION   Y13221
VERSION     Y13221.1  GI:2342561
KEYWORDS    glnA gene; glutamine synthetase I.
SOURCE      Corynebacterium glutamicum
ORGANISM    Corynebacterium glutamicum
Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales;
Corynebacterineae; Corynebacteriaceae; Corynebacterium.
REFERENCE   1
AUTHORS     Jakoby,M., Tesch,M., Sahn,H., Kramer,R. and Burkowski,A.
TITLE       Isolation of the Corynebacterium glutamicum glnA gene encoding
            glutamine synthetase I
JOURNAL     FEMS Microbiol. Lett. 154 (1), 81-88 (1997)
PUBMED      9237824
REFERENCE   2  (bases 1 to 3686)
AUTHORS     Jakoby,M.J.
TITLE       Direct Submission
JOURNAL     Submitted (16-MAY-1997) M.J. Jakoby, Forschungszentrum Juelich, IBT
            I, Postfach 1913, 52428 Juelich, FRG
FEATURES
     source          1..3686
                     /organism="Corynebacterium glutamicum"
                     /mol_type="genomic DNA"
                     /strain="ATCC 13032"
                     /db_xref="taxon:1718"
     gene            1274..2707
                     /gene="glnA"
     CDS             1274..2707
                     /gene="glnA"
                     /codon_start=1
                     /transl_table=11
                     /product="glutamine synthetase I"
                     /protein_id="CAA73664.1"
                     /db_xref="GI:2342562"
                     /db_xref="GQA:Q79VE3"
                     /db_xref="HSSP:POA590"
                     /db_xref="InterPro:IPR004809"
                     /db_xref="InterPro:IPR008146"
                     /db_xref="InterPro:IPR008147"
                     /db_xref="UniProtKB/TrEMBL:Q79VE3"
                     /translation="MAPETPEEIVKPKIDENVEFVDVVRPTDLPCTEQHFSIPAAASFDA
                     DTIEEGIAFDGSSIRGPTTIDESDMNLLPDLSTATLDPFRKAKTLNVKFFVHDPTRE

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AFSRDPRNVARKAHOYLASTGIACTCNFGAEAEFYLPDSVRYSTEMNNGSGFYEVIVTEEG
WNRUKETNLNDSTENLCAKVRKGVFPVAPYDQTVVDVDDVNLAAAGSPALERTHI
EVGSGQQEINVRFTMLHAADDITQTFKYITKVTARLHGKAAATFMPEKPLACDNGSGMHA
HQSLSWKDGPKEPHDESGYACLSIDTARYYIGLIHAGAVLAFTMTATLSYHRLVPGFE
AFINIVYSGQRNRSAJVRIPITGSGNPKAKRIEFRAPDPSGNYLGFAMMMAGLDGICKN
RIEPHAPVDKDLVELPEPEAASIPQAPTSLEASLKALQEDOTPLTESDVPTEDLIEAY
IQYKYDNEISVPVRLRPTPQEFELYDC"
stem_loop      2730..2769
                /note="terminator of glnA"
ORIGIN
Query Match      98.7%; Score 2466.4; DB 14; Length 3686;
Best Local Similarity 99.2%; Pred. No. 0;
Matches 2479; Conservative 0; Mismatches 21; Indels 0; Gaps 0;

Qy      1 CTCTGTGCGGGGACGAAAAATTTGCAACTCTCGCTTTGTCTAGCTAGATCAACCCCAACCA 60
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Db    401 CTCTGTGCGGGGACGAAAAATTTGCAACTCTCGCTTTGGCTAGCTAGATCAACCCCAACCA 460

Qy      61 AGCAGCAAGGGCGCTCGATCCCCGCAAAAGATCGGCGCCCATATAATTTCACTCAAGACAAAT 120
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Db    461 AGCAGCAAGGGCGCTCGATCCCCGCAAAAGATCGGCGCCCATATAATTTCACTCAAGACAAAT 520

Qy      121 TACCCGCGGATTAAC TGAGTTCCCGTTGCCTTTGCTGTGCTGGAGGCCACGGCGCTCAGCATCC 180
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Db    521 TACCCGCGGATTAAC TGAGTTCCCGTTGCCTTTGCTGTGCTGGAGGCCACGGCGCTCAGCATCC 580

Qy      181 ACCATCACGCGAGCGAGAAATCAAAATGGTCAAGTGGAGCAACAGCGCACGCCACCAA 240
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Db    581 ACCATCACGCGAGCGAGAAATCAAGATCGTCAAGTGGGCGAACAGCGCACGCCACCGAG 640

Qy      241 CCCACACGCTCCTCTGCAATCCACGCGCGAAGGCCCATGCCCCAACCGGCAATGACCTGGG 300
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Db    641 CCCACACGTTCTCTGCAATCCACGCGCGAAGGCCCATGCCCCAACCGGCAATGACCTGGG 700

Qy      301 GTGCGAGCAAAAGATCAATCCGTTAGCCAAACCCAGGATCAGCAAAATTAATGAGCGTGGAT 360
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Db    701 GTGCGAGCAAAAGATCCAACCGGTAAGCCAAACCCAGGATCAGCAAAATTAATGAGCGTGGAT 760

Qy      361 GTGCGTACATCGCGCAGCACATCGTGAAATTGGACAGCAATAGCAATAACCCAGGAA 420
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Db    761 GTGCGTACATCGCGCAGCACATCGTGAAATTGGACAGCAATAGCAATAACCCAGGAA 820

Qy      421 ACACCCCACTCCACGCGAGACCCCGCGATACGACGAGCCACTGAGGACAGAGAGCGCGCC 480
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Db    821 ACACCCCACTCCACGCGAGACCCCGCGATACGACGAGCCACTGAGGACAGAGAGCGCGCC 880

Qy      481 CTTTCTTTGAGGAAGCCCCAACTTTTTCGCCAGGCCACTGCCGGCGCATCAGGATCGTCA 540
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Db    881 CTTTCTTTGAGGAAGCCCCAACTTTTTCGCCAGGCCACTGCCGGCGCATCAGGATCGTCA 940

Qy      541 AAATCAGCTGGAATTTTCGGGTCCGTCAAGGCCAACTTCTCTTCGGGTTTGCATTTGTACA 600
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Db    941 AAATCAGCTGGAATTTTCGGGTCCGTCAAGGCCAACTTCTCTTCGGGTTTGCATTTGTACA 1000

Qy      601 ATCAAAATCCAAACATGTAGAGGGCGGATACCTGCGAGTCAAAAGGCGTTTGCCTTTAGAGCTC 660
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Db    1001 ATCAAAATCCAAACATGTAGAGGGCGGATACCTGCGAGTCAAAAGGCGTTTGCCTTTAGAGCTC 1060

Qy      661 GCAAAGCGCAATTTTCCTACCTTTAAGATCCTTAATCTGTTGAGGTCAGGCCAATTTTCA 720
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Db    1061 GCAAAGCGCAATTTTCCTACCTTTAAGATCCTTAATCTGTTGAGGTCAGGCCAATTTTCA 1120

Qy      721 GAAAAGTTTTGATAGATCGACAGGTAATGCATTATACCTGACAACTCGCGAAGGACTACAT 780
      | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Db    1121 GAAAAGTTTTGATAGATCGACAGGTAATGCATTATACCTGACAACTCGCGAAGGACTACAT 1180

Qy      781 TTGCAGCCAAAGTCTACTACTTGATCTTCAAAGGTCAGCAATTTGGAACAAAGCTACAAAT 840

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Db      1181  |||||
1181  TTGCAGCCAAAGTCTACTACTTGTATCTTCAAAGTTCAGCAATTGTGAAACAAAGTCACAAAT 1240
Qy      841  AAACCGTTCCACCCATGTCAATGAGGAGTCAACGTTGGCGTTTGAAACCCCGGAGAAATTT 900
Db      1241  AAACCGTTCCGCCCATGTCAATGAGGAGTCAACGTTGGCGTTTGAAACCCCGGAGAAATTT 1300
Qy      901  GTCAAAGTTCATCAAGGATGAAAACGTGAGTTGCTTGACGTTGATTTACCGACCTTCCTCC 960
Db      1301  GTCAAAGTTCATCAAGGATGAAAACGTGAGTTGCTTGACGTTGATTTACCGACCTTCCTCC 1360
Qy      961  GGCACCGGAGCAGCACTTTACGATCCGAGCTGCCAGCTTCGATGCAGATACAGTCGAAAGAA 1020
Db      1361  GGCACCGGAGCAGCACTTTACGATCCGAGCTGCCAGCTTCGATGCAGATACAAATCGAAAGAA 1420
Qy      1021  GGTCTCGCATTCGACGGATCTCGATCCGTGGCTTCACACGATCGACGAATCTGACATG 1080
Db      1421  GGTCTCGCATTCGACGGATCTCGATCCGTGGCTTCACACGATCGACGAATCTGACATG 1480
Qy      1081  AATCTCTCTGCGAGACCTTCGGAAACGGCCACCCCTTGATCCATTCCGCAAGGCAAAAGACCTTG 1140
Db      1481  AATCTCTCTGCGAGACCTTCGGAAACGGCCACCCCTTGATCCATTCCGCAAGGCAAAAGACCTTG 1540
Qy      1141  AACGTTAAGTTCTTCTGTTTACGATCCTTTTACCCGCGAGGCAATCTCCCGGACCCCAACGC 1200
Db      1541  AACGTTAAGTTCTTCTGTTTACGATCCTTTTACCCGCGAGGCAATCTCCCGGACCCCAACGC 1600
Qy      1201  AACGTAGCAGCAAGGCGAGAGCACTACCTGGCATCCACCGGCATTGCGAGACACCTGCAAC 1260
Db      1601  AACGTAGCAGCAAGGCGAGAGCACTACCTGGCATCCACCGGCATTGCGAGACACCTGCAAC 1660
Qy      1261  TTGGCGCGCGAGGCTGAGTTCTACTCTCTTGGACTCCGTTTGGCTACTCCACCGAGATGAAC 1320
Db      1661  TTGGCGCGCGAGGCTGAGTTCTACTCTCTTGGACTCCGTTTGGCTACTCCACCGAGATGAAC 1720
Qy      1321  TCCGCGCTTCTACGAAGTAGATACCGAAGAAGGCTGGTGAACCGTGGCAAGGAACCAAC 1380
Db      1721  TCCGCGCTTCTACGAAGTAGATACCGAAGAAGGCTGGTGAACCGTGGCAAGGAACCAAC 1780
Qy      1381  CTGCACGGAAACCCAAACCTGGGCGCAAGAACCGCGTCAAGGGTGGCTACTTCCAGTA 1440
Db      1781  CTGCACGGCAACCCAAACCTGGGCGCAAGAACCGCGTCAAGGGTGGCTACTTCCAGTA 1840
Qy      1441  GCACCATACGACCAACCGTTGACGTGCGCGATGACATGGTTGCGCAACCTCGACGCTTCC 1500
Db      1841  GCACCATACGACCAACCGTTGACGTGCGCGATGACATGGTTGCGCAACCTCGACGCTTCC 1900
Qy      1501  GGGTTGCTCTTTGAGCGTTTCCACCGAAGTCGTTGGCGGACAGCAGGAATCAACTAC 1560
Db      1901  GGGTTGCTCTTTGAGCGTTTCCACCGAAGTCGTTGGCGGACAGCAGGAATCAACTAC 1960
Qy      1561  CGCTTCAACACCATGCTCCACGCGGCGAGATGATATCCAGACCTTCAAGTACATCATCAAG 1620
Db      1961  CGCTTCAACACCATGCTCCACGCGGCGAGATGATATCCAGACCTTCAAGTACATCATCAAG 2020
Qy      1621  AACACCGCTCGCCTCCACGCGAAGGCTGCAACCTTCAATGCTTAAGGCACCTGGTGGCGAC 1680
Db      2021  AACACCGCTCGCCTCCACGCGAAGGCTGCAACCTTCAATGCTTAAGGCACCTGGTGGCGAC 2080
Qy      1681  AACGGTTCCGGCATGCACGCTCAACGATCCCTCTGGAAGGACGGCAAGCCACTCTTCCAC 1740
Db      2081  AACGGTTCCGGCATGCACGCTCAACGATCCCTCTGGAAGGACGGCAAGCCACTCTTCCAC 2140
Qy      1741  GATGAGTCCGGCTACGCGAGGCTGTCCGACATCGCCCGGTACTACATCGCGCGCATCTCTG 1800
Db      2141  GATGAGTCCGGCTACGCGAGGCTGTCCGACATCGCCCGGTACTACATCGCGCGCATCTCTG 2200
Qy      1801  CACCACGACGGCGCTGTTCTGGCGTTCAACCAACGCAACCTTGAACTCTACCAACGCTCTG 1860

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Db	2201		CACCACGCAAGCGCTGTTCTGGCGTTCAACAACGCAACCGTGAATCTCTACGACCGTCTG	2260
Qy	1861		GTTCCAGGCTTCGAGGCTCCAAATCAACCTGGTGTACTACAGCGCAACCGTTCGGCTGGT	1920
Db	2261		GTTCCAGGCTTCGAGGCTCCAAATCAACCTGGTGTACTACAGCGCAACCGTTCGGCTGGT	2320
Qy	1921		GTCGCTATCCCAATCACCGGATCCAAACCGAAAGCGCAAGCGCATCGAATTCGGGCTCCA	1980
Db	2321		GTCGCTATCCCAATCACCGGATCCAAACCGAAAGCGCAAGCGCATCGAATTCGGGCTCCA	2380
Qy	1981		GACCCATCAGSCAACCCATACCTGGGCTTCGACGCGATGATGATGGCGGCTTCGACGGC	2040
Db	2381		GACCCATCAGSCAACCCATACCTGGGCTTCGACGCGATGATGATGGCGGCTTCGACGGC	2440
Qy	2041		ATCAAGAACCGCATCGAGCCACAGCTCCAGTGGACAAGGACCTCTACAGACTGCCACCA	2100
Db	2441		ATCAAGAACCGCATCGAGCCACAGCTCCAGTGGACAAGGACCTCTACAGACTGCCACCA	2500
Qy	2101		GAGGAAGCTGCATCCATTCCACAGGCACCAACCTCCCTGGAAGCATCCCTGAAGGCACTG	2160
Db	2501		GAGGAAGCTGCATCCATTCCACAGGCACCAACCTCCCTGGAAGCATCCCTGAAGGCACTG	2560
Qy	2161		CAGGAAGACACCGACTTCTCTACCGAGTCTGACGTCTTCACCGAGGATCTCATCGAGGGC	2220
Db	2561		CAGGAAGACACCGACTTCTCTACCGAGTCTGACGTCTTCACCGAGGATCTCATCGAGGGC	2620
Qy	2221		TACATCCAGTACAAGTACGACAACGAGATCTCCCGAGTTTCGCCCTGCGGCCAACCCGCGAG	2280
Db	2621		TACATCCAGTACAAGTACGACAACGAGATCTCCCGAGTTTCGCCCTGCGGCCAACCCGCGAG	2680
Qy	2281		GAATTCGAATTGTACTTCGACTGCTAAATTCACCTTAGCTAGCCGATAGCGGAAACCCCGTG	2340
Db	2681		GAATTCGAATTGTACTTCGACTGCTAAATTCACCTTAGCTAGCCGATAGCGGAAACCCCGTG	2740
Qy	2341		AAATTCCTCATTTGAATTCAGGGGGTTTCTTTTTTACATTCCACCTAAAAGGAAAGCGCC	2400
Db	2741		AAATTCCTCATTTGAATTCAGGGGGTTTCTTTTTTACATTCCACCTAAAAGGAAAGCGCC	2800
Qy	2401		GGATCCTCCCATCATGGTGGATCCGCGGCTTTATCTATTGTTTTTTGGGCTAGATGCCGA	2460
Db	2801		GGATCCTCCCATCATGGTGGATCCGCGGCTTTATCTATTGTTTTTTGGGCTAGATGCCGA	2860
Qy	2461		TCAGTTCAGATGCAACTACATCGGACAGTGAGACGGTTCC	2500
Db	2861		TCAGTTCAGATGCAACTACATCGGACAGTGAGACGGTTCC	2900

Pompejus et al. teach a *C. glutamicum* strain and a gene from that *C. glutamicum* strain encoding glutaminase (Table 1, page 56, Glutamate and Glutamine metabolism, RXA00335 and RXN03176; SEQ ID NO: 101-102). The nucleotide sequence of the glutaminase gene of Pompejus et al. has been disclosed as SEQ ID NO: 101 in Pompejus et al. and is 861 nucleotides long. An alignment of this polynucleotide with the polynucleotide of SEQ ID NO: 1 shows that this sequence is 99% sequence identical to nucleotides 827-1687 of SEQ ID NO: 1 (99% = 851x100/861). A copy of this alignment (included in the Office action mailed on 3/5/2007) is provided below.

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RESULT 5
 AAF71803
 ID AAF71803 standard; DNA; 861 BP.
 XX
 AC AAF71803;
 XX
 DT 30-APR-2001 (first entry)
 XX
 DE **Corynebacterium glutamicum MP protein nucleotide sequence SEQ ID NO:101.**
 XX
 KW Corynebacterium glutamicum; metabolic pathway protein; MP protein;
 KW fine chemical production; microorganism; organic acid; nucleoside;
 KW nonproteinogenic amino acid; purine base; pyrimidine base; nucleotide;
 KW lipid; saturated fatty acid; unsaturated fatty acid; diol; vitamin;
 KW carbohydrate; aromatic compound; cofactor; polyketide; enzyme; ds.
 XX
 OS Corynebacterium glutamicum.
 XX
 PN **WO200100843-A2.**
 XX
 PD **04-JAN-2001.**
 XX
 PF 23-JUN-2000; 2000WO-IB000923.
 XX
 PR 25-JUN-1999; 99US-0141031P.
 PR 01-JUL-1999; 99DE-01030476.
 PR 02-JUL-1999; 99US-0142101P.
 PR 08-JUL-1999; 99DE-01031415.
 PR 08-JUL-1999; 99DE-01031418.
 PR 08-JUL-1999; 99DE-01031419.
 PR 08-JUL-1999; 99DE-01031420.
 PR 08-JUL-1999; 99DE-01031424.
 PR 08-JUL-1999; 99DE-01031428.
 PR 08-JUL-1999; 99DE-01031434.
 PR 08-JUL-1999; 99DE-01031435.
 PR 08-JUL-1999; 99DE-01031443.
 PR 08-JUL-1999; 99DE-01031453.
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 PR 08-JUL-1999; 99DE-01031478.
 PR 08-JUL-1999; 99DE-01031510.
 PR 08-JUL-1999; 99DE-01031541.
 PR 08-JUL-1999; 99DE-01031573.
 PR 08-JUL-1999; 99DE-01031592.
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 PR 08-JUL-1999; 99DE-01031636.
 PR 09-JUL-1999; 99DE-01032125.
 PR 09-JUL-1999; 99DE-01032126.
 PR 09-JUL-1999; 99DE-01032130.
 PR 09-JUL-1999; 99DE-01032186.
 PR 09-JUL-1999; 99DE-01032206.
 PR 09-JUL-1999; 99DE-01032227.
 PR 09-JUL-1999; 99DE-01032228.
 PR 09-JUL-1999; 99DE-01032229.
 PR 09-JUL-1999; 99DE-01032230.
 PR 14-JUL-1999; 99DE-01032922.
 PR 14-JUL-1999; 99DE-01032926.
 PR 14-JUL-1999; 99DE-01032928.
 PR 14-JUL-1999; 99DE-01033004.
 PR 14-JUL-1999; 99DE-01033005.
 PR 14-JUL-1999; 99DE-01033006.
 PR 12-AUG-1999; 99US-0148613P.
 PR 27-AUG-1999; 99DE-01040764.
 PR 27-AUG-1999; 99DE-01040765.
 PR 27-AUG-1999; 99DE-01040766.
 PR 27-AUG-1999; 99DE-01040832.
 PR 31-AUG-1999; 99DE-01041378.

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PR 31-AUG-1999; 99DE-01041379.
 PR 31-AUG-1999; 99DE-01041380.
 PR 31-AUG-1999; 99DE-01041394.
 PR 31-AUG-1999; 99DE-01041396.
 PR 03-SEP-1999; 99DE-01042076.
 PR 03-SEP-1999; 99DE-01042077.
 PR 03-SEP-1999; 99DE-01042079.
 PR 03-SEP-1999; 99DE-01042086.
 PR 03-SEP-1999; 99DE-01042087.
 PR 03-SEP-1999; 99DE-01042088.
 PR 03-SEP-1999; 99DE-01042095.
 PR 03-SEP-1999; 99DE-01042124.
 PR 03-SEP-1999; 99DE-01042129.
 PR 09-MAR-2000; 2000US-0187970P.
 XX
 FX (BADI) BASF AG.
 XX
 FI **Pompejus M, Kroegeer B, Schroeder H, Zelder O, Haberhauer G;**
 XX
 DR WFI; 2001-137957/14.
 DR P-PSTDB; AAB79684.
 XX
 PT Nucleic acids from *Corynebacterium glutamicum* encoding metabolic pathway
 PT proteins, useful for producing fine chemicals in microorganisms,
 PT including organic acids, nonproteinogenic amino acids, and purine and
 PT pyrimidine bases.
 XX
 PS Claim 3; Page 314-315; 1737pp; English.
 XX
 CC AAF71753 to AAF72330 encode the *Corynebacterium glutamicum* metabolic
 CC pathway (MP) proteins given in AAB79634 to AAB80211. The C. glutamicum MP
 CC nucleic acids are useful for the production of fine chemicals in
 CC microorganisms, including organic acids, nonproteinogenic amino acids,
 CC purine and pyrimidine bases, nucleosides, nucleotides, lipids, saturated
 CC and unsaturated fatty acids, diols, carbohydrates, aromatic compounds,
 CC vitamins, cofactors, polyketides and enzymes
 XX
 SQ Sequence 861 BP; 199 A; 309 C; 205 G; 148 T; 0 U; 0 Other;

Query Match 40.2%; Score 845; DB 4; Length 861;
 Best Local Similarity 98.8%; Pred. No. 3.4e-214;
Matches 851; Conservative 0; Mismatches 10; Indels 0; Gaps 0;

Qy 827 GAGTTGGCCGATTACATCCCGGAACATAAAATCTGCGGACCCAAACCCGCTGGCAGTAGCC 886
 |||||
 Db 1 GAGTTGGCCGATTACATCCCGGAACATAAAATCTGCGGACCCAAACCCGCTGGCAGTAGCC 60

Qy 887 CTGTGCAACGGTTAACGSGACACATCTACAGCGCAGGCGATGACGACATCGAAATTCACCATG 946
 |||||
 Db 61 CTGTGCAACGGTTAACGSGACACATCTACAGCGCAGGCGATGACGACATCGAAATTCACCATG 120

Qy 947 CAAAGTATTTCOAAGCCCTTTGGCTACGCACTCGCACTCCAAAGATGGGGTTTGATGAG 1006
 |||||
 Db 121 CAAAGTATTTCOAAGCCCTTTGGCTACGCACTCGCACTCCAAAGATGGGGTTTGATGAG 180

Qy 1007 GTCTCTGCATCCGTGGCCTTTGGAACCCCTCCGGTGAAGGCTTCACAGAACTTCCCTCGAG 1066
 |||||
 Db 181 GTCTCTGCATCCGTGGCCTTTGGAAGCCCTCCGGTGAAGGCTTCACAGAACTTCCCTCGAG 240

Qy 1067 GCGAANAACCGCCCATGAACCCCATGATCAACGCGCGCGGATCGCCATCAACACGCTG 1126
 |||||
 Db 241 GCGAANAACCGCCCATGAACCCCATGATCAACGCGCGCGGATCGCCATCAACACGCTG 300

Qy 1127 ATCAACGGCTCCGACATCCACCGTGAAGAGACGAGTGGAAAAATCCGACACTACTTCTCT 1186
 |||||
 Db 301 ATCAACGGCTCCGATTCACACCGTGAAGAGACGCGTGGAAAAATCCGACACTACTTCTCT 360

Qy 1187 GAACCTGTGGAAGCGCACTCAACATCGACCGCGTGCTTGGCAATCCGAACTCGCGCGG 1246

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Db      361  |||ACTTTGTTGGACGGAACTCACCATGACCGGCTGCTTGCGGAATCGGAACTCGCGGGC 420
Qy      1247  GCCGACCGCAACCTCTCCATCGGCCACATGCTGCGCAACTATGGCGTCATCGAAGACGAA 1306
Db      421  GCCGACCGCAACCTCTCCATCGGCCACATGCTGCGCAATTACGGCGTCATCGAAGACGAA 480
Qy      1307  GCCACGAGCGCGCTCTCAGCTACACGCTGCAATGTGCGCATCAAGTAACACACGGCGGAC 1366
Db      481  GCCACGAGCGCGCTCTCAGCTACACGCTGCAATGTGCGCATCAAGTAACACACGGCGGAC 540
Qy      1367  CTGCGAGTCATGACCGCACGCTCGCGCGCGGCGGCAACCCAAATTACCGGCAAGAAG 1426
Db      541  CTGCGAGTCATGACCGCACGCTCGCGCGCGGCGGCAACCCAAATTACCGGCAAGAAG 600
Qy      1427  CTTCTCGACGCGCGGCTGTGCGGCTCACCCTCTCCGTCATGGCTTCAGCAGGCAATGTAC 1486
Db      601  CTTCTCGACGCGCGGCTGTGCGGCTCACCCTCTCCGTCATGGCTTCAGCAGGCAATGTAC 660
Qy      1487  GACGAGGCAAGGCGAGTGGCTCTCCACCGTAGGCAATCCCCCGGAATACAGGAGTCGCGGGC 1546
Db      661  GACGAGGCAAGGCGAGTGGCTCTCCACCGTAGGCAATCCCCCGGAATACAGGAGTCGCGGGC 720
Qy      1547  GSACATCATCGGCATTCTGCCAGGTGAGCTGGGCAATCGCCACATTTTCCCAAGCCTGAAC 1606
Db      721  GSACATCATCGGCATTCTGCCAGGTGAGCTGGGCAATCGCCACATTTTCCCAAGCCTGAAC 780
Qy      1607  CCCAAGGCAAACAGCGTGCAGCGGCGTAAAAATATTCAAACAGCTTTCCGACGACATGGGC 1666
Db      781  CCCAAGGCAAACAGCGTGCAGCGGCGTAAAAATATTCAAACAGCTTTCCGACGACATGGGC 840
Qy      1667  CTCACCTTATGTCCACCGAG 1687
Db      841  CTCACCTTATGTCCACCGAG 861

```

In view of the fact that the specification discloses that the gene encoding the *C. glutamicum* glutaminase of SEQ ID NO: 2 is 2100 nucleotides long, one would reasonably expect the glutaminase gene in the *C. glutamicum* of Pompejus et al. to have a nucleotide sequence which is longer than that disclosed originally by Pompejus et al. (longer than SEQ ID NO: 101 of Pompejus et al.) by virtue of being from the same organism, i.e., *C. glutamicum*, and at the same locus since the percent identity of SEQ ID NO: 101 with a fragment of SEQ ID NO: 1 is extremely high (99%). While Pompejus et al. do not disclose the entire sequence of their glutaminase gene, Nakagawa et al. provides the remaining structure of the *C. glutamicum* glutaminase gene of Pompejus et al. (GenBank accession number AX127151; from EP 1108790). This is shown in an alignment of SEQ ID NO: 101 from Pompejus et al. against chromosomal DNA from *C. glutamicum* taught by Nakagawa et al. (GenBank accession number AX127151) where SEQ ID NO: 101 from Pompejus et al. is completely comprised by the chromosomal

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/mol_type="genomic DNA"
/db_xref="taxon:1718"
/noFe="Seq 1 to long (3.309.400) split in 11, seq 7067
2.400.001 2.749.980"

ORIGIN

Query Match          95.4%; Score 2004.4; DB 2; Length 349980;
Best Local Similarity 97.6%; Pred. No. 0;
Matches 2059; Conservative 0; Mismatches 41; Indels 10; Gaps 2;

Qy      1  CACAAAAATCCGSGCAATCCACCGAAATCGTCTTCATCTTTGGCTTGATCAAATGCCTCAT 60
Db      228498 CACAAAAATCCGSGCAATCCACCGAAATCGTCTTCATCTTTGGCTTGATCAAATGCCTCAT 228439

Qy      61  TCGGCCCGGCTGCACCGTCACGCTTTCGAGAAATAGAAATAGCGCTTGTGCAGCCACCC- 119
Db      228438 TCGGCCCGGCTGCACCGTCACGCTTTCGAGAAATGAAATAGCGCTTGTGCAGCCACCCCT 228379

Qy      120  -----CACTCTCAACGCGACGCGCGAGCGGTGGCATCAGCCAGGATTTATTAGGA 171
Db      228378 CAACGCGAGCCGCGACGAGCGCTGTGCCAGCGGTGGCATCAGCCAGGATTTATTAGGA 228319

Qy      172  CCGGCGATATAGGTAAATGGAGTGGCAGCCCTGATCCACCAATGGACCAACAGCCTTCGCC 231
Db      228518 CCGGCGATATAGGTAAATGGAGCGGCGACCCCTGATCCACCAATGGACCAACAGCCTTCGCC 228259

Qy      232  GTACCGTCGTAGTTATCCACCATCAGCTTGGGAATACCTTGCACTTCACGCTCATTAAT 291
Db      228258 GCACCGTCGTAGTTATCCACCATCAGCTTGGGAATACCTTGCACTTCACGCTCATTAAT 228199

Qy      292  ACAGTGGGAATTTCCCGCGCGACTTTTGGGATCTCACCAGAAATCCATCCCTGAAGCAGCG 351
Db      228198 ACAGTGGGAATTTCCCGCGCGACTTTTGGGATCTCACCAGAAATCCATCCCTGAAGCAGCG 228139

Qy      352  AGCAATAAGCCATCGCGGTGGGGAGCATCTTTGTCCAGCACTCCCTGGACTTAATCGCC 411
Db      228138 AGCAATAAGCCATCGCGGTGGGGAGCATCTTTGTCCAGCACTCCCTGGACTTAATCGCC 228079

Qy      412  GACTCCCGGGGCTCGACAAGCGCAACCGTATAGCCCTGAGTGCCTTGGGGCATGCTGGCGG 471
Db      228078 GACTCCCGGGGCTCGACAAGCGCAACCGTATAGCCCTGAGTGCCTTGGGGCATGCTGGCGG 228019

Qy      472  CCTGGAAAAATTTCCAAAGAAGGGAATCGATGCATCGGTGGCAACCATAGCATGATA 531
Db      228018 CCGCGAAAAATTTCCAAAGAAGGGAATTCGACGATCGCGGGCAACCATAGCATGAGG 227959

Qy      532  CGGCTGTTTTGGGCGGTGAAAAGCGCTGAGTTTCCACACGCGTTGCGGATTTTCTCCGCGAT 591
Db      227958 CGGCTGTTTTGGGCGGTGAAAAGCGCTGAGTTTCCACACGCGTTGCGGATTTTCTCCGCGAT 227899

Qy      592  GGAAGAACTCACTCGGCCAGGCTTCGAAAAAGCGCGCGGACACAGTGGAAAGGGAGACGCC 651
Db      227898 GGAAGAACTCACTCGGCCAGGCTTCGAAAAAGCGCGCGGACACAGTGGAAAGGGAGACGCC 227839

Qy      652  AGCGACTTTTGGGCAATCATAAATGGTGGCTTTTGAGTCGCTGTG-GCCCGAGAACTCTGT 710
Db      227838 AGCGACTTTTGGGCAATCATAAATGGTGGCTTTTGAGTCGCTGTGAGGCCCGAGAACTCTGT 227779

Qy      711  CATGCACAAAGATATATAGCGCAAAAGAAATCACTAGTCTTGATTTCTATGTTCAGCATGC 770
Db      227778 CATGCACAAAGATATATAGCGCAAAAGAAATCACTAGTCTTGATTTCTATGTTCAGCATGC 227719

Qy      771  CGATACCGGAGTACCTGCACGAAATTTTAGATGATGTCCGCGACACCACTCCGCGAGT 830
Db      227718 CGATACCGGAGTACCTGCACGAAATTTTAGATGATGTCCGCGACACCACTCCGCGAGT 227659

Qy      831  TGGCCGATTTACATCCCGGAACATAAAATCTGCCGACCCAAACCGCGTGGCGATAGCCCTGT 890
Db      227659 TGGCCGATTTACATCCCGGAACATAAAATCTGCCGACCCAAACCGCGTGGCGATAGCCCTGT 227600

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Db	227658	TGCGGATTACATCCCGGAACATAAAATCTTGGGACCCAAACCGCTGGCGATAGCCCTGT	227599
Qy	891	GCACCGTTAACGGACACATCTACACGGCAGCGGATGACGACATCGAATTCACCATGCAAA	950
Db	227598	GCACCGTTAACGGACACATCTACACGGCAGCGGATGACGACATCGAATTCACCATGCAAA	227539
Qy	951	GTAATTCCAAAGCCCTTTGGCTACGCACTCGCACTCCAGAAATGGGGTTTGATGAGGTCT	1010
Db	227538	GTAATTCCAAAGCCATTTGGCTACGCACTCGCACTCCAGAAATGGGGTTTGATGAGGTCT	227479
Qy	1011	CTGCATCCGTGSCCTTGGAAACCTTCGGGTGAGSCCTTCAACGAACCTTCCCTCGACGGG	1070
Db	227478	CTGCATCCGTGSCCTTGGAAACCTTCGGGTGAGSCCTTCAACGAACCTTCCCTCGACGGG	227419
Qy	1071	AAAACGCCCCCATGAACCCCATGATCAAGCGCGGCGGATCGCCATCAACAGCTGATCA	1130
Db	227418	AAAACGCCCCCATGAACCCCATGATCAAGCGCGGCGGATCGCCATCAACAGCTGATCA	227359
Qy	1131	ACGGCTCCGACTCCACGCTGGAAGACCGAGTGGAAAAAATCCGACACTACTTCTCTGAAC	1190
Db	227358	ACGGCTCCGATTCCACGCTGGAAGACCGGCTGGAAAAAATCCGACACTACTTCTCTGAAC	227299
Qy	1191	TTGCTGGACGGGAACTGACCATCGACGGGTGCTTGCGGAATCCGAATCGCGGGGCGG	1250
Db	227298	TTGCTGGACGGGAACTGACCATCGACGGGTGCTTGCGGAATCCGAATCGCGGGGCGG	227239
Qy	1251	ACCGCAACCTTCCATCGCCACATGCTGCGCACTATGGCTCATCGAAGACGAAGCC	1310
Db	227238	ACCGCAACCTTCCATCGCCACATGCTGCGCAATTACGGGCTCATCGAAGACGAAGCC	227179
Qy	1311	ACGACGCGCTCTCAGGTACACGCTGCAATGTGCCATCAAAGTAACACGCGGACCTCG	1370
Db	227178	ACGACGCGCTCTCAGGTACACGCTGCAATGTGCCATCAAAGTAACACGCGGACCTCG	227119
Qy	1371	CAGTCATGACGCGCACGCTCGCGCGCGGGGACGCAACCAATACCGGCAAGAAGCTTC	1430
Db	227118	CAGTCATGACGCGCACGCTCGCGCGCGGGACACACCAATACCGGCAAGAAGCTTC	227059
Qy	1431	TCGACGCGCGGCTTCTGCGGCTCACCTCTCTCGTCAATGGCTTCAGCAGGATGTACGACG	1490
Db	227058	TCGACGCGCGGCTTCTGCGGCTCACCTCTCTCGTCAATGGCTTCAGCAGGATGTACGACG	226999
Qy	1491	AGGACGGGAGTGGCTCTCCACCGTAGGCATCCCGCGGAATCAGGAGTCCGCGGGGAC	1550
Db	226998	AGGACGGGAGTGGCTCTCCACCGTAGGCATCCCGCGGAATCAGGAGTCCGCGGGGAC	226939
Qy	1551	TCATCGGCATTCTGCGAGGTACAGCTGGGCATGCGCCACATTTTCCCGACGCTGAACCCCA	1610
Db	226938	TCATCGGCATTCTGCGAGGTACAGCTGGGCATGCGCCACATTTTCCCGACGCTGAACCCCA	226879
Qy	1611	AAGGCAACAGCGTGGCGGCGGTAAAAATATTCAAACAGCTTTCCGACGACATGGGCTCC	1670
Db	226878	AAGGCAACAGCGTGGCGGCGGTAAAAATATTCAAACAGCTTTCCGACGACATGGGCTCC	226819
Qy	1671	ACCTTATGTCCACGAGCAGGTATCCGGGACGCAAGTACGATCATTCAGCGGGACGGG	1730
Db	226818	ACCTTATGTCCACGAGCAGGTATCCGGGACGCAAGTACGATCATTCAGCGGGACGGG	226759
Qy	1731	ACACCACTTTCATCCAAATGACGGGCGCCATGAACCTTCTCAGCCAGCAAGAGCTTCTCC	1790
Db	226758	ACACCACTTTCATCCAAATGACGGGCGCCATGAACCTTCTCAGCCAGCAAGAGCTTCTCC	226699
Qy	1791	ACGCCATCGTGGAAACACAACTTTGAAGGCACGGAAGTTGTTCTTGATCTCACCGAGTAC	1850
Db	226698	ACGCCATCGTGGAAACACAACTTTGAAGGCACGGAAGTTGTTCTTGATCTCACCGAGTAC	226639
Qy	1851	TTAGCTTCCACCCCGTAGCCATCCGATGATCAAGAGGCGCTCAAAAGCATCCGCGACG	1910

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Db      226638  TTAGCTTCACCCCGTAGCCATCCGCATGATCAAGAAGGCGCTCAAAAGCATCCGCGACG  226579
Qy      1911  CAGGCTTTGAGGTGTTTCATCCTCGACCCAGATGACGTACTGCCCGATTTCATGTTTTCGG  1970
      |||
Db      226578  CAGGCTTTGAGGTGTTTCATCCTCGACCCAGATGACGTACTGCCCGATTTCATGTTTTCGG  226519
Qy      1971  ACGGCACCATCTGCAAAAGAACGAGTGTGACCGGTAGCTTTATGGTCTGAACAATTGCGAAG  2030
      |||
Db      226518  ACGGCACCATCTGCAAAAGAACGAGTGTGACCGGTAGCTTTATGGGTGAACAATTCTAAG  226459
Qy      2031  GAGATTAATCGGTGAAAAAGAACGCTTATGTTGCCCTTTGATTGTGCAGCTTTGGGATTAA  2090
      |||
Db      226458  GAGAAATTATCCGTGAAAAAGAACGCTTATGTTGCCCTTTGATTGTGCAGCTTTGGGGTTAA  226399
Qy      2091  GTGCCTGCAG  2100
      |||
Db      226398  GTGCCTGCAG  226389

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Therefore, even if one were to interpret the term “95% or more homologous to SEQ ID NO: 1/3” as “95% or more sequence identical to the polynucleotide of SEQ ID NO: 1/3, (1) the *C. glutamicum* of Pompejus et al., as evidenced by Nakagawa et al., comprises an endogenous glutaminase gene which is more than 95% sequence identical to the polynucleotide of SEQ ID NO: 1, and (2) the glutamine synthetase gene of Jakoby et al. is at least 95% sequence identical to the polynucleotide of SEQ ID NO: 3. As such, the structural limitations recited in the claims with regard to the glutaminase and glutamine synthetase genes are met by the teachings of the cited art. Pompejus et al. also teach that the disclosed *C. glutamicum* gene can be used for the modulation of production of amino acids (page 11, lines 20-25) and that glutamine is used in both pharmaceutical and cosmetics industries (page 13, lines 17-19). Pompejus et al. do not teach a mutant coryneform bacterium wherein the glutaminase activity in said bacterium has been reduced and glutamine synthetase activity has been enhanced.

Claims 1, 4-5, 13-16, 19-21 are directed in part to a coryneform bacterium that produces L-glutamine modified such that (1) the glutaminase activity of said bacterium is reduced by disrupting the endogenous glutaminase gene on the chromosome, and the glutamine synthetase activity in said bacterium is increased by increasing the copy number of a gene encoding a glutamine synthetase or by placing said gene under the control of the lac, trp, or trc promoter, wherein the glutaminase gene to be disrupted is a structural variant of the polynucleotide of SEQ ID NO: 1 that hybridizes under the stringent conditions recited in claim 1 to the polynucleotide of SEQ ID NO: 1, and the glutamine synthetase gene

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is a structural variant of the polynucleotide of SEQ ID NO: 3 that hybridizes under the stringent conditions recited in claims 5 and 16 to the polynucleotide of SEQ ID NO: 3, wherein the glutamine synthetase activity in said bacterium is at least double that of the glutaminase activity, and wherein said glutaminase activity is 0.01 U/mg protein or less.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the *C. glutamicum* cell that endogenously comprises the *C. glutamicum* glutaminase gene of Pompejus et al. by (1) deleting all or most of the coding region of the glutaminase gene, and (2) increasing the expression of the *C. glutamicum* glutamine synthetase gene of Jakoby et al. either by increasing its copy number or by placing said gene under the control of the lac, trp, or trc promoters, for the benefit of producing L-glutamine in view of the fact that glutaminase catalyzes the degradation of L-glutamine and glutamine synthetase catalyzes the formation of L-glutamine. A person of ordinary skill in the art is motivated to construct such *C. glutamicum* cell in view of the fact that (1) Duran et al. teach an increase in L-glutamine production when the glutaminase gene is disrupted, (2) Pompejus et al. teach that L-glutamine is a chemical used in the pharmaceutical and cosmetics industries, (3) Duran et al. teach that glutaminase degrades L-glutamine to glutamate, (4) Nakamura et al. teach a method for the production of L-glutamine where a reduction in the production of L-glutamic acid is desired, (5) Nakamura et al. teach that increasing glutamine synthetase activity results in an increase in L-glutamine production, and (6) the use of strong heterologous promoters allows for controlled expression of the protein of interest as they require the presence of inducers for expression to occur (e.g., lactose and tryptophan).

One of ordinary skill in the art has a reasonable expectation of success at modifying such *C. glutamicum* cell in view of the fact that (1) Pompejus et al. teach the *C. glutamicum* cell comprising the endogenous glutaminase gene recited in the claims, (2) Jakoby et al. teach the *C. glutamicum* glutamine synthetase gene recited in the claims, (3) inactivation of genes by introducing deletions/insertions if the sequence of the target gene is known is well known and widely practiced in the art, (4) Nakamura et al.

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teach increased expression of the glutamine synthetase gene for increased L-glutamine production in *C. glutamicum*, (5) Duran et al. teach that inactivation of the glutaminase gene results in increased L-glutamine production, and (6) increased expression by increasing the copy number of the gene of interest and the use of lac, trc, or trp promoters is well known in the art. In the absence evidence to the contrary, if no additional sources of glutaminase activity are present, a deletion of the glutaminase gene wherein most or all of the coding region is removed would result in no glutaminase activity (i.e., 0 U/mg protein). If the glutaminase activity is 0 U/mg protein, then the glutamine synthetase activity would be expected to be at least double that of glutaminase. Therefore, the invention as a whole would have been prima facie obvious to a person of ordinary skill in the art at the time the invention was made.

(10) Response to Argument

A. Legal Standard

On pages 4-7, Appellant refers to case law which Appellant indicates as relevant to the issues of indefiniteness, written description, enablement and obviousness. The Examiner acknowledges the case law cited by Appellant.

B. The rejection of claims 1, 4-5, 12-16, and 18-21 under 35 U.S.C. §112 2nd paragraph is in error

On pages 8-10, Appellant argues that the Examiner's position ignores established precedent in Group 1600 and refers to patents issued to Appellant from Art Unit 1652 where similar language to that used in claims 1, 5 and 16 (i.e., "95% or more homologous to SEQ ID NO: X") is present. Appellant argues that the reason such language is universally accepted as being clear and definite is not found in the specification but rather in the standardized and well-practiced methods for determining homology between sequences, citing BLAST as a computer algorithm commonly used in the art for calculating homology. With regard to claims 12 and 18, Appellant argues that these claims should be excluded

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from the rejection because the genus of genes recited is defined as one which encompasses genes that encode the polypeptide of SEQ ID NO: 2, thus being a very definite group of genes and essentially nullifying the 95% homology language of the independent claim. Appellant further argues that it is agreed that the terms "homology" and identity are not the same and that Appellant chose to claim "95% homologous" and not "95% identity", thus it is impermissible that the Examiner would construe the claims in any other way. Appellant also argue that the Examiner is incorrect when asserting that the specification does not provide the specific parameters/methods intended in the calculation of sequence homology because the Examiner must also look beyond the specification and the knowledge of the art for the definition of this term. It is appellant's contention that calculations for determining homology have been made for decades, and that while they may give varying results, such variances can be accounted for and recognized by virtue of the fact that these calculations have been routinely made for a long time.

Appellant's arguments have been fully considered but not found persuasive. With regard to arguments referring to a "established precedent" in Group 1600 or the cited US patents, it is noted that each application is examined on its own merits and any discussion regarding policy or previously issued US patents would be improper herein in view of the fact that the Examiner has no authority to comment in regard to policy, and any discussion of other applications would require review of the record of each individual case. While one could have interpreted the term "95% or more homologous to SEQ ID NO: X" to be limited to "95% or more sequence identical to SEQ ID NO: X", it is abundantly clear from Appellant's responses, including the Brief, that the term "homology" should not be read as "identity". The Examiner has acknowledged throughout the prosecution of the instant application that methods to calculate homology, including BLAST, are known in the art. It is reiterated herein that the issue in the instant application is not whether one of skill in the art would not know how to calculate homology. The issue in the instant case is whether one of skill in the art can determine the scope of the claims when there is a specific numerical value recited (95%), there are different ways to calculate homology that would

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render different homology values for a particular set of sequences, and neither the claim nor the specification provides a clue as to which is the intended method to calculate such homology value so that it would be clear as to which species fall within the genus of nucleic acids recited and which ones are excluded from the recited genus.

With regard to claims 12 and 18, it is reiterated herein that if the claims were to encompass the entire genus of genes encoding the protein of SEQ ID NO: 2, the instant rejection would not be applicable to these claims. Instead, claims 12 and 18 encompass a subset of the genus of genes encoding the protein of SEQ ID NO: 2, wherein said genes hybridize under the conditions recited to the polynucleotide of SEQ ID NO: 1 and are at least 95% sequence homologous to SEQ ID NO: 1. In view of the fact that it is unclear which of the genes that hybridize under the conditions recited fall within the scope of “at least 95% sequence homologous to SEQ ID NO: 1”, it follows that it is unclear as to which of the genes recited in claim 1b) are encompassed by the subset of genes of claims 12 and 18.

Arguments regarding the knowledge of the art with regard to variations in the calculation of homology and how these variations are accounted for, recognized, and not an issue with regard to indefiniteness are not persuasive because the issue is not whether or not one would recognize or account for the variability in the calculation of homology, but rather which is appellant's intended scope for the term “95% or more homologous to SEQ ID NO: X”. As previously stated, using the scoring table (matrix) IDENTITY_NUC and parameter values Gapop = 10.0 and Gapext = 1.0, the polynucleotide of GenBank accession number BA000036_26 is 95.4 % sequence homologous to the polynucleotide of SEQ ID NO: 1. If the same calculation is carried out with the same scoring table and parameter values Gapop = 10.0 and Gapext = 0.1, the polynucleotide of GenBank accession number BA000036_26 is 96.1 % sequence homologous to the polynucleotide of SEQ ID NO: 1. If the claim were to recite “96% or more homologous to SEQ ID NO: 1”, could the polynucleotide of GenBank accession number BA000036_26 be considered as encompassed by the term “96% or more homologous to SEQ ID NO:

1”)? There is no definite answer to that question because there are several ways to calculate homology and the specification is silent with regard to how the calculation should be made. In the instant case, the claims include a numerical limitation without (1) stating which of the multiple methods/parameters for calculating homology should be used, or (2) providing some guidance as to how to determine which polynucleotides are encompassed/excluded by the term. As such, one of skill in the art cannot reasonably conclude that the term “95% or more homologous to SEQ ID NO: X” is clear and definite.

C. The rejection of claims 1, 4-5, 12-16, 18, 19, and 21 under 35 U.S.C. §112 1st paragraph, written description, is in error

On pages 10-12, Appellant argues that a “precise reduction” is not required by the claims but a reduction to any level below 0.1 U/mg. Similarly, Appellant argues that the ratio of activities of claims 4 and 15 do not require the skilled art worker to achieve exactly the 2:1 ratio of glutamine synthetase to glutaminase activity. Appellant asserts that the Examiner is incorrect in asserting that the values recited for activity and ratio can be obtained by any means because the claims require that the glutaminase gene be disrupted or mutated. According to Appellant, disruption and/or mutation of genes are well known procedures in the art. Appellant further refers to Exhibit A, which is an alignment of six sequences corresponding to glutaminases, and states that one of skill in the art can recognize from this alignment which are the important regions associated with enzymatic activity and determine where to introduce amino acid mutations to reduce activity. Appellant argues that one of skill in the art can also use the sequence information for glutaminase genes, determine the regulatory regions, and mutate/disrupt said regulatory regions. Thus, Appellant is of the opinion that one of skill in the art can obtain a coryneform bacterium as claimed where glutaminase activity is reduced to 0.1 U/mg of cellular protein or any level below this activity level. In addition, Appellant refers to Exhibit B, which is an alignment of glutamine synthetase genes (gln) from *B. flavum*, *C. glutamicum*, *C. efficiens* and *M. tuberculosis*, and asserts that the gln genes are highly conserved. Therefore, one of skill in the art would have reasonably understand

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that a DNA sequence which has 95% or more homology to SEQ ID NO: 3 would naturally encode a protein having glutamine synthetase activity.

Appellant's arguments have been fully considered but are not deemed persuasive. As clearly stated in the claims and contrary to Appellant's assertions, the claims do require the precise levels of glutaminase activity (0.1 and 0.01 U/mg) as well as the precise ratio of glutaminase to glutamine synthetase activity (1:2) in view of the recitation of "glutaminase activity of the bacterium is reduced to 0.1 U/mg of cellular protein or less", "glutaminase activity of the bacterium is reduced to 0.01 U/mg of cellular protein or less", and "glutaminase activity is $\frac{1}{2}$ or less than glutamine synthetase activity" (emphasis added). While it is agreed that disruption of the glutaminase gene, either within the regulatory or coding regions, would result in no glutaminase activity (0 U/mg), and 0 U/mg protein is a species of the genus of levels of glutaminase activity recited, the claims (1) encompass more than this level of glutaminase activity, and (2) also recite "mutating a glutaminase gene" as the method to achieve the recited 0.1/0.01 U/mg. While disruption is one of the mutations that can be made to a gene, the term "mutating a glutaminase gene" encompasses structural modifications in the regulatory region of the gene as well as structural modifications in the coding region of the gene such that the encoded protein would have the recited enzymatic activity. The mutations which would result in a glutaminase activity of 0.1 or 0.01 U/mg of cellular protein have not been described in the specification nor the prior art. Since the recited levels are the main embodiments of the genus of glutaminase activity levels recited, one of skill in the art would expect, at a minimum, that the specification would adequately describe those embodiments which are considered representative of the genus.

Appellant's arguments regarding how one of skill in the art can use the alignment of six sequences corresponding to glutaminases to determine which are the structural modifications that would lead to the desired glutaminase levels are not found persuasive because (1) not all conserved regions are necessarily related to activity and can be an artifact of how evolutionarily related the sources of the

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glutaminases are - the more closely related two organisms are, the more structural conservation one would expect between proteins having the same function; (2) conserved regions alone without some structure/function correlation won't provide guidance/suggestion as to the effect of making a particular modification on enzymatic activity levels (e.g., would making a substitution at position X within conserved region Y result in a reduction to Z U/mg protein?), and (3) conserved regions are dependent on the sequences which are used in the alignment and there is no indication that the conserved regions in the alignment provided are found in glutaminases not used in the alignment provided by Appellant.

With regard to the recited ratio of glutaminase to glutamine synthetase activity, it is reiterated herein that the claims do not place any limitations regarding the modifications that can be made to the bacterium to adjust glutamine synthetase activity and meet the desired ratio. It is noted that the ratio is a function of both enzymatic activities. As stated in previous Office actions, some of the claims encompass not only increasing the expression of the DNA of SEQ ID NO: 3 and the structural variant of the DNA of SEQ ID NO: 3 as recited in claims 5 and 16, but they also encompass increasing the activity of any glutamine synthetase activity (from any organism) in said bacterium to achieve the recited ratio by any means. As such, the modifications encompass not only structural modifications in the regulatory or coding regions of the endogenous glutamine synthetase genes, but also the expression of unknown transcription modulators of glutamine synthetase genes as well as the addition of chemical inducers of transcription or chemical enhancers of enzymatic activity.

The Examiner has previously acknowledged the alignment of glutamine synthetase amino acid sequences provided. However, as previously stated, conserved regions in an alignment are sequence-dependent, and in the absence of any teaching or suggestion that the conserved regions in an alignment are also going to be found in other glutamine synthetases, it is unclear as to whether the conserved regions found within a limited number of sequences are related to activity. Appellant's arguments that one of skill in the art would reasonably conclude that the recited structural variants of the polynucleotide

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of SEQ ID NO: 3 would naturally encode a glutamine synthetase are not persuasive in view of the fact that the art, as evidenced by Seffernick et al. and Witkowski et al., clearly teaches that even when two proteins share a substantial amount of structural features (98% sequence identity in Seffernick et al., or one single amino acid substitution in Witkowski et al.), their activities are not the same. Furthermore, there is no teaching in the art suggesting that all the structural variants of the polynucleotide of SEQ ID NO: 3 as recited that do not encode the protein of SEQ ID NO: 4 would encode proteins having glutamine synthetase activity, nor there is any structure/function correlation disclosed. As previously indicated, even if the assumption is made that the claims encompass variants of the polynucleotide of SEQ ID NO: 3 having at least 95% sequence identity to the polynucleotide of SEQ ID NO: 3, the genus of proteins encoded by that genus of polynucleotides will include proteins which are 74% sequence identical to the polypeptide of SEQ ID NO: 4 (see calculation above). Thus, contrary to Appellant's assertions, one of skill in the art cannot reasonably conclude that all the recited variants of the polynucleotide of SEQ ID NO: 3 would encode a protein having glutamine synthetase activity.

D. The rejection of claims 1, 4-5, 12-16, 18, and 18-21 under 35 U.S.C. §112 1st paragraph, enablement, is in error

On pages 12-14, Appellant reiterates the arguments previously stated regarding the written description issue. Appellant argues that a "precise reduction" is not required by the claims but a reduction to any level below 0.1 U/mg. Similarly, Appellant argues that the ratio of activities of claims 4 and 15 do not require the skilled art worker to achieve exactly the 2:1 ratio of glutamine synthetase to glutaminase activity. Appellant asserts that the Examiner is incorrect in asserting that the values recited for activity and ratio can be obtained by any means because the claims require that the glutaminase gene be disrupted or mutated. According to Appellant, disruption and/or mutation of genes are well known procedures in the art. Appellant states that it is illogical to find the end point of 0 U/mg protein enabled but the range of 0.1-0.01 U/mg not. Also, Appellant cites case law in support of the argument that

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inoperable embodiments are permitted to be encompassed by the claims and that the exemplification of every embodiment is not required to satisfy the requirements of 35 U.S.C. §112 1st paragraph. Appellant refers to Exhibits A and B as described above and reiterates that (1) one of skill in the art could recognize from this alignment which are the important regions associated with glutaminase activity and determine where to introduce amino acid mutations to reduce activity, (2) one of skill in the art can also use the sequence information for glutaminase genes, determine the regulatory regions, and mutate/disrupt said regulatory regions, (3) one of skill in the art can obtain a coryneform bacterium as claimed where glutaminase activity is reduced to 0.1 U/mg of cellular protein or any level below this activity level, (4) gln genes are highly conserved in view of the alignment of Exhibit B, and (5) one of skill in the art would conclude that a DNA sequence which has 95% or more homology to SEQ ID NO: 3 would naturally encode a protein having glutamine synthetase activity.

Appellant's arguments have been fully considered but are not deemed persuasive. The Examiner has already addressed those arguments which were first provided with regard to the written description rejection. See extensive discussion above. With regard to the argument that it is illogical to find the end point of 0 U/mg protein enabled but the range of 0.1-0.01 U/mg not, Appellant is reminded that the instant situation is analogous to having one species in the genus enabled while the remainder of the genus not enabled. As such, there is nothing illogical in finding one of the species of the genus of glutaminase levels enabled but not others. As previously stated, the claims are enabled for a disruption in the glutaminase gene which would result in no enzymatic activity. However, neither the specification nor the prior art teach or suggest how to achieve the precise glutaminase activity levels or the specific ratio of glutaminase to glutamine synthetase activity recited in the claims. As discussed above, in addition to requiring any type of mutation to the glutaminase gene to achieve the recited glutaminase levels, the claims also require any type of modification to achieve the recited ratio of glutaminase to glutamine synthetase activity. These modifications can be mutations, as well as the expression of unknown

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transcription inducers or the addition of chemical transcription inducers or chemicals which enhance glutamine synthetase activity. Determining which mutations and modifications out of any number of possibilities is not considered routine in the art. With regard to inoperable embodiments, while the examiner agrees that inoperable embodiments are permitted to be encompassed by the claims and that the exemplification of every embodiment is not required to satisfy the requirements of 35 U.S.C. § 112 1st paragraph, in the instant case there is only one embodiment that has been enabled for the recited genus of mutations and glutaminase activities, which is disruption of the glutaminase gene to eliminate glutaminase activity (0 U/mg), and two embodiments that have been enabled for the recited genus of modifications and recited ratio, which is increasing the copy number of the glutamine synthetase gene or replacing the promoter of said glutamine synthetase gene with a stronger heterologous promoter. Thus, one of skill in the art would not reasonably conclude that these embodiments enable the entire claimed invention.

E. The rejection of claims 1, 4-5, 13-16, and 19-21 under 35 U.S.C. §103

On pages 14-16, Appellant argues that one of skill in the art would have not been motivated or have any reason to combine the teachings of the cited references. Specifically, Appellant argues that Duran et al. only teach uptake of glutamine added to the medium by bacterial cells and does not show production of glutamine by the LM16 strain in the culture medium. Appellant refers to Table 1 of Duran et al. and states that the intracellular glutamate level in the LM16 strain is low compared to that in the wild type strain when the mutant is cultivated in a medium containing ammonium and succinate as growth substrates. Appellant states that glutamine is synthesized from glutamate and ammonium in a reaction catalyzed by glutamine synthetase, thus one of skill in the art would know that the production of glutamine decreases when the intracellular glutamate concentration decreases. Appellant further argues that Figure 3 of Duran et al. shows that the amount of ¹⁴CO₂ released from the LM16 strain and the wild-type strain are almost the same when the cells are cultivated in a medium containing glutamine and

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succinate, thus it is appellant's contention that one of skill in the art would not conclude from the teachings of Duran et al. that knocking out glutaminase would result in increased glutamine productivity since glutaminase is clearly not involved, or involved very minimally, in the degradation of glutamine.

Appellant's arguments have been fully considered but are not found persuasive. It appears from Appellant's remarks there is no dispute with regard to the teachings of the cited art as they relate to the genes recited in the claims. The Examiner has acknowledged that Duran et al. do not teach extracellular glutamine levels or a coryneform bacterium. However, as previously indicated before, Duran et al. clearly teach that by reducing glutaminase activity, there is an increase in glutamine levels. The abstract of the reference by Duran et al. is reproduced below:

The role of glutaminase in *Rhizobium etli*: studies with a new mutant

Socorro Durán, Gisela Du Pont, Alejandra Huerta-Zepeda
and Jorge Calderón

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In order to examine the role of glutaminase in *Rhizobium etli*, we isolated and characterized a *R. etli* glutaminase mutant (LM16). This mutant was selected for its impaired ability to grow on glutamine as nitrogen and carbon source while retaining the ability to grow on other nitrogen and carbon sources. The mutant showed very low levels of glutaminase activity under various growth conditions in comparison with the wild-type strain. With glutamine as the only nitrogen and carbon source, LM16 showed poor growth, with a very high content of glutamine, low glutamate content, and reduced ammonium excretion and $^{14}\text{CO}_2$ evolution from $[\text{U-}^{14}\text{C}]\text{glutamine}$ compared to the wild-type strain. This indicates that the main role of *R. etli* glutaminase is in the use of glutamine as carbon source. *R. etli* glutaminase also plays a role in maintaining the balance between glutamate and glutamine, as shown by the accumulation of glutamine and the low glutamate content of the mutant under different growth conditions. These results also indicate that glutaminase participates in a glutamine cycle in which it degrades glutamine which is then resynthesized by glutamine synthetase. The higher glutamine and lower glutamate content found in bacteroids of LM16 in comparison with bacteroids of the wild-type strain indicate that glutamine degradation by glutaminase plays an important role during the symbiosis between *R. etli* and *Phaseolus vulgaris*.

As clearly stated by Duran et al., glutaminase is an enzyme which catalyzes the degradation of glutamine. Therefore, any suggestion that glutaminase is not involved in the degradation of glutamine is contrary to the knowledge of one of skill in the art. In addition, the conclusion that a reduction in glutaminase activity results in an increase in glutamine levels is explicitly stated by Duran et al.,

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presumably based on their own data. Therefore, it is unclear to the Examiner as to how one of skill in the art could reasonably conclude from the teachings of Duran et al. that knocking out glutaminase would not result in increased glutamine productivity, or that glutaminase is clearly not involved in the degradation of glutamine. Table 1 of the reference by Duran et al. is presented below for the reader's convenience:

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Table 1. Glutamine and glutamate content of *R. etli* wild-type strain and LM16 mutant grown on different substrates

Amino acid content was determined after 5 h growth and is expressed as nmol (mg protein)⁻¹.

Growth substrate	Wild-type		LM16	
	Glutamine	Glutamate	Glutamine	Glutamate
Glutamine	0.9	21.5	48.0	6.6
Glutamine + succinate	0.6	27.5	3.3	15.6
Ammonium + succinate	0.4	16.1	0.9	5.5
Peptone+yeast extract	0.4	22.3	0.8	18.8

Appellant has argued that the level of glutamate in the mutant LM16 (has reduced glutaminase activity) is lower than the level of glutamate in the wild-type, therefore one of skill in the art would know that the production of glutamine decreases when the intracellular glutamate concentration decreases. In response, Appellant's attention is directed to every single glutamine entry for the wild-type and LM16 strains, where it is clear that for all the growth substrates used, the levels of glutamine are higher in the glutaminase deficient mutant. Even if one argues that the levels of glutamine are higher when glutamine is externally added as growth substrate, the increase in glutamine levels is double for those cases where no glutamine was externally added as growth substrate (from 0.4 to 0.9/0.8). See the results obtained with ammonium+succinate and peptone+yeast extract.

Appellant has argued that in view of the teachings of Figure 3 regarding the amount of ¹⁴CO₂ released from the LM16 strain and the wild-type strain when the cells are cultivated in a medium, one of skill in the art would not conclude from the teachings of Duran et al. that knocking out glutaminase would result in increased glutamine productivity since glutaminase is clearly not involved, or involved very

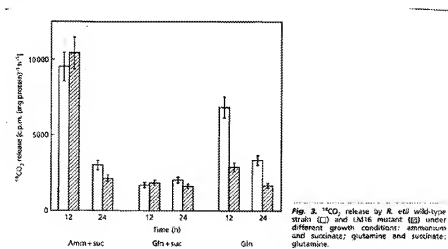
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minimally, in the degradation of glutamine. In response, Appellant's attention is directed to pages 2886-2887 of the reference by Duran et al. where it is stated:

To evaluate the participation of glutaminase in the utilization of glutamine as carbon source, we measured the $^{14}\text{CO}_2$ released from $[\text{U-}^{14}\text{C}]$ glutamine by the LM16 mutant and wild-type strain under different growth conditions. Fig. 3 shows that, compared with the wild-type strain, the mutant liberated similar amounts of $^{14}\text{CO}_2$ ($\text{mg protein}^{-1} \text{ h}^{-1}$) when it was grown on ammonium plus succinate or on glutamine plus succinate, whereas on

glutamine as nitrogen source, the mutant liberated twofold lower amounts of $^{14}\text{CO}_2$ ($\text{mg protein}^{-1} \text{ h}^{-1}$) than the wild-type strain.

A copy of Figure 3 is reproduced below:



Thus, it is clear from the teachings of Duran et al. that the amount of $^{14}\text{CO}_2$ released was measured to determine the role of glutaminase in the utilization of glutamine as a carbon source and not as an indicator of how glutamine productivity is affected by reducing glutaminase activity. Thus, contrary to Appellant's assertions, one of skill in the art would not interpret the results of $^{14}\text{CO}_2$ release when the substrate is glutamine and succinate as an indication that glutaminase is not involved (or minimally involved) in the degradation of glutamine.

On pages 16-17, Appellant argues that the observation that intracellular glutamine levels are increased when glutamine is used as a carbon source is likely because glutamine is being imported into the cell and not being degraded due to the lack of glutaminase activity. Furthermore, Appellant argues that when succinate and glutamine are added to the medium, intracellular glutamine levels decreased as compared to when glutamine was used as the sole carbon source. Appellant indicates that the strain of Duran et al. is not believed to have the ability to produce glutamine and argues that one of skill in the art would have reasonably concluded that the glutamine level in bacterial cells would decrease based on the teachings of Nakamura et al., Duran et al., Jakoby et al. and Pompejus et al. Appellant argues that the claims are directed to a coryneform bacterium which has L-glutamine producing ability, which according to the specification is an ability to accumulate L-glutamine in a medium, and that such bacterium is not disclosed or suggested by Duran or the other cited references.

Appellant's arguments have been fully considered but not deemed persuasive. Appellant's attention is directed to the glutamine levels found in the LM16 mutant and the wild-type strain when ammonium+succinate and peptone+yeast extract were used (Table 1). In those cases, no additional glutamine was externally added, yet the level of glutamine was double in the glutaminase deficient strain (from 0.4 to 0.9/0.8). While it is agreed that the increase in glutamine levels varies with the substrate used, the overall effect of reduced glutaminase activity on glutamine levels was the same in all cases, an increase in glutamine levels. It should be noted that the claims do not have any limitation as to how much glutamine should be produced by the bacterium, thus the issue of whether the increase in glutamine levels is small or large is not relevant to the instant case.

With regard to arguments that Duran et al. do not teach an L-glutamine producing organism, it is noted that while it is acknowledged that the strain of Duran et al. is not considered a high L-glutamine producer such as the *C. glutamicum* strain taught by Pompejus et al. or Nakamura et al., it meets the definition given in the specification for an L-glutamine producing organism in view of the fact that this

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bacterium will produce L-glutamine and some L-glutamine will accumulate in the growth medium partly due to cell lysis. It should be noted that the definition provided in the specification does not place any limitations on how much L-glutamine has to accumulate in the medium for the organism to be considered an L-glutamine producer. Also, as indicated above and in previous Office actions, the Examiner has not relied on Duran et al. for meeting the limitation “coryneform bacterium having L-glutamine-producing ability” as required by the claims. Instead, the Examiner has relied upon the teachings of Pompejus et al. or Nakamura et al. for meeting the “coryneform bacterium” limitation.

On pages 17-18, Appellant argues that the bacterium of Duran et al. is known to have an enzyme called GOGAT which catalyzes the formation of glutamic acid from glutamine in the presence of NADPH and does not function in the absence of α -ketoglutarate (α KG). Appellant attributes the glutamine accumulation shown in Table 1 when glutamine is used as the sole carbon source to the lack of α KG, which then results in a non-functional GOGAT. Appellant indicates that simultaneous addition of succinate and glutamine lead to a marked decrease in intracellular glutaminase levels compared to when glutamine is the only carbon source, and that this decrease is due to the activity of GOGAT as succinate addition results in an increase of α KG produced via the TCA cycle. Appellant points out that glutamine fermentation is usually carried out in the presence of glucose and that such fermentation would result in accumulation of α KG. Thus, appellant asserts that GOGAT is expected to have a greater contribution to the degradation of glutamine when glucose is added to the medium, as compared to when succinate is added to the medium. Appellant submits that Duran et al. do not teach or suggest an increase in glutamine level in a glutaminase-deficient bacterium when glucose is present in the medium and asserts that Duran et al. teach away from the claimed invention because in the presence of glucose, glutamine levels decrease. As such, Appellant concludes that there is no reason or motivation to combine the cited references to arrive to the claimed invention.

It is reiterated herein that it is agreed that degradation of glutamine is not carried out only by glutaminase since glutamate synthetase (GOGAT) would also use glutamine as a substrate. However, even if one takes into account the possibility of other enzymes also degrading glutamine, this fact would not lead one of skill in the art to conclude that it is unlikely that inactivating glutaminase would not result in some reduction in glutamine degradation. While the teachings of Duran et al. suggest that growth substrate is a factor in how much glutamine accumulation is obtained, the effect of reduced glutaminase activity on glutamine levels was found to be the an increase in glutamine levels in all cases considered.

The Examiner disagrees with the argument that Duran et al. teach away from using a glutaminase-deficient strain for accumulation of glutamine if the strain is grown in the presence of glucose, in view of the fact that (1) there is no teaching or suggestion in Duran et al. indicating that no glutamine accumulation is expected in the presence of glucose, (2) there is no experimental evidence by Appellant or in the prior art showing that the strain of Duran et al., when grown in the presence of glucose, would provide absolutely no glutamine accumulation, and (3) there is no evidence that glutaminase will be inactivated by the presence of glucose or its activity be impaired by products from glucose metabolism. Even if it is assumed that the contribution of GOGAT in glutamine degradation would be higher if the strain is grown in glucose, it is unlikely that the lack of glutaminase activity would have no effect whatsoever on glutamine levels. It should also be noted that there is no limitation in the claims regarding the presence or absence of glucose in any medium used to cultivate the claimed bacterium, thus whether glucose is used or not in the cultivation of the claimed bacterium is irrelevant to the discussion. The teachings of Duran et al. clearly suggest that there is an effect on glutamine levels when glutaminase is inactivated. Therefore, the teachings of the art, as evidenced by Duran et al., make the inactivation of glutaminase not only obvious to try but also provide a reasonable expectation of observing some glutamine accumulation.

If the objective is to produce glutamine, one of skill in the art would reasonably conclude that one possible scheme to increase glutamine production is to enhance synthesis of glutamine and reduce degradation of glutamine. The teachings of Nakamura et al. provide the enhancement in the synthesis of glutamine in *C. glutamicum* (coryneform bacterium) by teaching how to increase the concentration of glutamine synthetase, which is an enzyme that catalyzes the formation of glutamine, whereas the results of Duran et al. would not only provide motivation, but would also provide the skilled artisan with a reasonable expectation of some glutamine accumulation as a result of inactivating glutaminase. As previously indicated, there is no limitation in the claims as to how much glutamine should be produced by the claimed bacterium. Therefore, for the reasons extensively discussed above and those in previous Office actions, the claimed invention is deemed obvious in view of the teachings of Nakamura et al., Duran et al., Jakoby et al. and Pompejus et al.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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